Development and Evaluation of Novel Herbal Formulation for Immunomodulatory and Galactagogue Activity

SUMMARY FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

PHARMACEUTICAL SCIENCES

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INTRODUCTION AND STATEMENT OF PROBLEM:

Global health observatory data published by WHO (World Health Organization) in 2020, 5.18 million children under age five died at the rate of 14,000 every day. Out of all under-five deaths 75% occurred within the first year of life and 47% occurred during the first month of life, respectively.Child Malnutrition is a major cause of deaths in children (approx. 45%) under the age of 5 years. [1]According to WHO's study, wasted children under the age of 5 years are 47 million, severely wasted are 14.3 million, and stunted are 144 million. The countries with low- and middle-income are mostly affected. [2]

Malnutrition:

Malnutrition causes involve many factors like individual level factors, socio-culture factors, food care and health factors, past medical history, family history and frequency of breast feeding. UNICEF's programs help build the foundations of good nutrition and prevent and treat all forms of malnutrition. [3]

Incidences of hyogalactia ranges from 23% to 65% within the first four months postpartum, which is progressively rising. For instance, in the USA only 49.4% of infants receive any breast milk by 6 months and just 26.7% are breast fed for full year. In the first month of life 2.4 million new-borns died worldwide in 2020. Out of total deaths under the age of 5 years, half (47%) of the deaths in 2020 occurred in neonatal period (first 28 days). Children under the age of 5 years, in 2020, 45 million were wasted and 149 million were stunted. The condition is severe in underdeveloped and undeveloped countries. 45% of child deaths are associated with malnutrition.[45]

Major cause of child death recorded to be following:

- Preterm complications
- Respiratory disease
- Diarrhea
- Other infections

UNICEF has mentioned the foundation of good nutrition as enlisted below: [3]

- To improve mother's nutrition during the pregnancy and prenatal and postnatal nutrition of newborns,
- To promote and support exclusive breastfeeding for the first 6 months of a child's life, and continue breastfeeding to age 2 or beyond,

- To facilitate timely, safe, appropriate, and high-quality complementary foods and
- To provide appropriate micronutrient interventions.

Statement of the problem

There is need to find suitable solution for nursing mothers which can enhance secretion of milk with higher immunoglobulin level and helps in improving disease resistance of child.

Possible Remedy

To develop a formulation for instant transfer of bioactive constituents

Outcome

- Enhance milk secretion in preterm-term delivery cases,
- Overcome initial dys-galactorrhea,
- Boost immunity of child

Literature review:

1. Shatavari

Biological source: Shatavari consists of dried roots of the plant*Asparagus racemosus*, belonging to family Asparagaceae.

Roots are silver, white, or ash coloured externally and white internally. They are spindle shaped with size varying from 5 to 15 cm * 2cm.

Bioactive Chemicals: Steroidal saponins, known as Shatvarins. Shatvarins- I to VI are present. Kaempferol along with Sarsapogen is isolated from woody portions of tuberous roots.

Activity of the drug: Galactagogue, tonic and diuretic. Also used in Rheumatism and nervine disorders.[6]

2. Guduchi

Biological source: Guduchi consists of dried stems of the climber *Tinospora cordifolia*, belonging to family Menispermaceae.

The stems are greyish black in colour. They are fibrous, cylindrical in shape and are 3-5 cm in length.

Bioactive constituents: It consists of Tinosporine, tinosporic acid, berberine and cordifol in sufficient amounts.

Activity of drug: The drug is used in general debility, pyrexia, and skin diseases. It is now gaining importance as a potent Immune booster.[7]

3. Fenugreek

Biological source: Fenugreek consists of dried seeds of *Trigonella foenum-graecum*, belonging to family Fabaceae.

Bioactive constituents: Seeds are rich in galactomannan. The mature seeds contain amino acids, carotene, vitamins and saponins such as Diosgenin and its glycosides, gitogenin, vitexin. The glycosides trigonelline, choline, kaempferol luteolin and quercetin are also found in seeds.

Activity of drug: The seeds are mucilaginous, demulcent, carminative, tonic, Galactagogue, astringent, antidiabetic and antihyperlipidemic. Also used in enlargement of liver and spleen, rheumatism, and anaemia.[6]

CONCEPTUAL FRAMEWORK AND RATIONALE OF THE STUDY

1. Selection of plants and its rationale
    a. In ancient literature the plant reported to have both galactagogue and immunomodulatory potential
    b. Used in traditional Ayurvedic formulations
2. Selection of animal model and its rationale
    a. Most suitable and data can be extrapolated
    b. Gives data up to second generation
3. Selection of delivery system for lactating women and its rationale
    a. Instant delivery of bioactive content
    b. Easy to prepare
    c. Easy to handle, store and carry
    d. Cost effective

OBJECTIVES

- To prepare/procure plant extracts and standardize the extracts.
- To design and develop formulation-effervescent tablet and granules.
- To perform biological evaluation of extracts for galactagogue and immunomodulatory potential.

Plan of work:

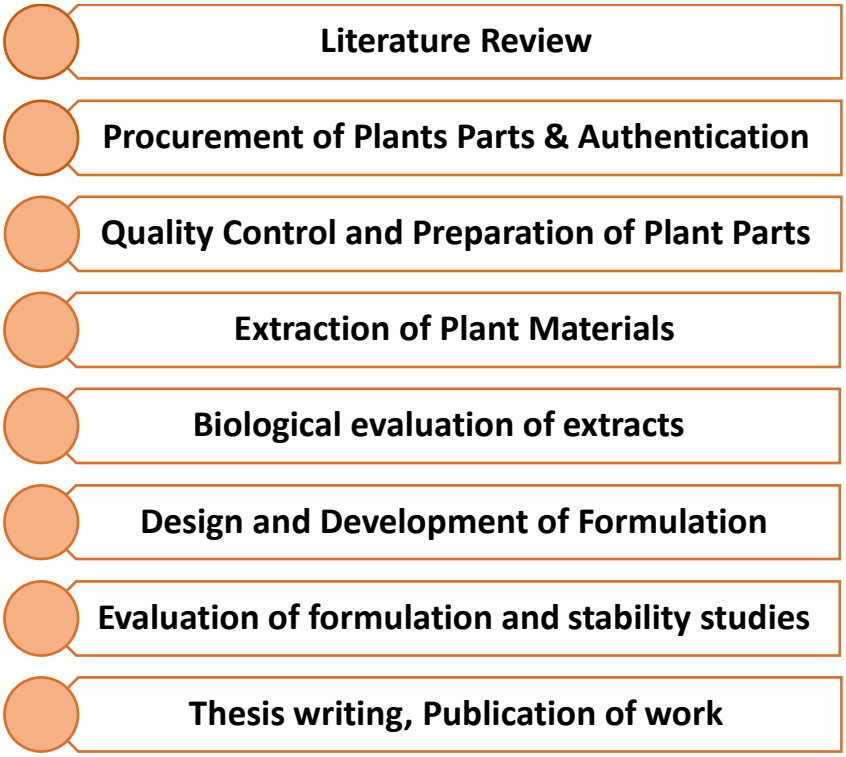

Literature Review
Procurement of Plants Parts & Authentication
Quality Control and Preparation of Plant Parts
Extraction of Plant Materials
Biological evaluation of extracts
Design and Development of Formulation
Evaluation of formulation and stability studies
Thesis writing, Publication of work

Figure 1. Plan of Work

Materials and Methods

The plant material [Dried roots of *Asparagus racemosus* (drug A), dried stems of *Tinospora cordifolia* (drug B), dried seeds of *Trigonella foenum-gracecum* L. (drug C)] were procured from D. G. Ayurvedic Sangrah, Andheri, Mumbai and authenticated from Department of Botany, Govt. Holkar Science College, Indore.

Metoclopramide and domperidone were procured from Cipla, Ltd., India.

Shatavarin IV (CAS No.84633-34-1) from Phyto Lab GmbH & Co., Germany, Trigonelline (CAS No.6138-41-6) from Sigma Aldrich, Switzerland and Berberine (CAS No. 5956-60-5) from Merck, Germany.

Preparation and standardization of extract:

Asparagus racemosus Extract

About 100gm roots of *Asparagus racemosus* were dried and powdered. The powdered drug was then extracted using Soxhlet apparatus with 1000 mL of 95 % ethanol at 55ºC. Under the reduced pressure (Vacuum) and temperature the extracts were concentrated and finally dried.[8]

Tinospora cordifolia Extract

The shed dried stems of *T. cordifolia* were coarsely powdered and extracted using Soxhlet apparatus with 95% ethanol. A semi-solid residue was obtained by vacuum drying of the filtrate at 55°C (vacuum evaporator).[9]

Trigonella foenum-gracecum Extract

The defatted fenugreek seeds were extracted with ethanol by Soxhlet apparatus. The extract was concentrated and dried under the vacuum and stored in a desiccator.[10]

The extract was standardised with HPTLC analysis against the analytical standards Shatavarin IV, Trigonelline and Berberine, for detecting presence of the Shatavarin IV, Trigonelline, Berberine, following parameters were selected in HPTLC analysis: Mobile Phase: n-butanol: Acetic acid: Water (14:3:4) v/v/v.

Stationary Phase: HPTLC Silica Gel 60 F254, Merck, Detecting Agent: Anisaldehyde-Sulphuric acid reagent, Solubility: Methanol, (Rf Value= solute run/solvent run)

Toxicity Studies

1. **Acute Toxicity Studies (14 days, as per OECD Guidelines Test Guideline 423)**

   In acute toxicity testing of drugs, healthy albino mice were divided into three groups (n=3) in which group 1 received purified water, group 2 received drug mixture 300 mg/kg, and group 3 received drug mixture 2000 mg/kg drug mixture, by oral route. For 14 days, animals were daily observed and recorded body weight, behaviour, and water and food intake. At the end of study animals were sacrificed and vital organ histopathology was performed.[16]

2. **Repeat Dose Toxicity Studies (28 days, as per OECD Guidelines Test Guideline 407)**

   In 28 days, repeated dose toxicity studies, animals were divided into four groups each consisting of 5 males and 5 females. Group 1 received purified water, while other received 250 mg/kg, 500 mg/kg, and 1000 mg/kg body weight of drug mixture by oral route for 28 days. During the study, daily behaviour, body weight, food and water intake was recorded. Last day of study, urine was collected and studies. At the end of study, the animals were sacrificed; blood was collected to record biochemical and

haematological changes. 10 organs were isolated from one animal from each group, weighed and their microscopic evaluation was done and compared with that of control group.[17]

Homology studies

The sequence alignment was done using The National Center for Biotechnology Information (NCBI), The Basic Local Alignment Search Tool (BLAST) and PyMol was used to visualize the structural alignment.

Lactation study

The study used 66 nursing New Zealand white rabbits (lactating does) weighing between 2.0 and 2.3 kg. The does were chosen when they had just started to nurse their litter of five kits. All rabbit studies were duly approved by the Institutional Animal Ethics Committee (IAEC) with an approval number IAEC/2019/01/01. The animals were maintained under standard laboratory conditions with free access to food and water. The experiment was performed according to the guidelines of the CCSEA (Committee for Control and Supervision of Experiments on Animals), Government of India. All the does were split into eleven groups, six animals in each group. The Group 1 (Control) received saline, Group 2 (allopathic drug) received domperidone- 1.54mg/kg BW po, Group 3 (allopathic drug) received metoclopramide- 1.54mg/kg BW po and Group 4 (herbal drug) received Shatavari extract- 150mg/kg BW po, Group 5 (herbal drug) received Shatavari extract- 300mg/kg BW po, Group 6 (herbal drug) received Guduchi extract- 150mg/kg BW po, Group 7 (herbal drug) received Guduchi extract- 300mg/kg BW po, Group 8 (herbal drug) received Fenugreek extract- 150mg/kg BW po, Group 9 (herbal drug) received Fenugreek extract- 300mg/kg BW po, Group 10 (herbal drug) received blend of extracts- 150mg/kg BW po, Group 11 (herbal drug) received blend of extracts - 300mg/kg BW po. The study started on the very 1st day of parturition. Does and kits weight were recorded daily before and after nursing for 21 days. Does milk and blood were collected on day 0, day 7, day 14, and day 21. The difference in body weight of kits before and after feeding was used to represent the amount of lactation. The amount of milk production was accumulated for each day. The cumulative data of six animals were recorded and processed for further evaluation. [11]

Table 1. Study Groups of Animals for Lactation study

S. no.	Drug	Group	No. of animals
1.	Control	E- Vehicle control	6
2.	Domperidone	1.54mg/kg	6
3.	Metoclopramide	1.54mg/kg	6
4	Drug A (*Asparagus racemosus*)	A1- Drug (Low dose- 150mg/kg)	6
5		A2- Drug (High dose- 300mg/kg)	6
6	Drug B (*Tinospora cordifolia*)	B1- Drug (Low dose- 150mg/kg)	6
7		B2- Drug (High dose- 300mg/kg)	6
8	Drug C (*Trigonella foenum graceum*)	C1- Drug (Low dose- 150mg/kg)	6
9		C2- Drug (High dose- 300mg/kg)	6
10	Drug A + B + C	D1- Drug blend- 1:1:1 (Low dose- 150mg/kg)	6
11		D2- Drug blend- 1:1:1 (High dose- 300mg/kg)	6
Total			66

Milk yield (MY) and analysis of milk composition (MC)

As a measure of MY, the mean of the doe's and kits' weight before and after suckling was taken during the three weeks of lactation (peak of milk production). The milk sucked by each kit is calculated by taking the difference in weight of kits after and before feeding. After 3 minutes of injecting does with 0.5 ml of oxytocin hormone to induce milk ejection, milk samples from each group were manually obtained for milk composition (MC) records by gently stroking the mammary gland. Quantitative analysis of milk nutrients was done by automated Lactoscan Milk Analyzer (Milkotronic Ltd., Bulgaria). The amount of protein, fat, and lactose in rabbit milk was determined. Milk total minerals were calculated as total ash value calculated using the AOAC method. The ash content was estimated by igniting the dried samples in a muffle furnace at 460^0C, as instructed by Association of Official Analytical Chemist.[12]

Serum Prl Estimation

Just after delivery and at the end of every week of the lactation study, the blood was collected from the marginal ear vein; serum was separated, and the Prl was estimated by using electrochemiluminescence (Rabbit Prl ELISA kit, Catalog Number RPRL.96, MD biosciences, USA).[13]

Histopathology of mammary glands

On the 22nd day, does were anesthetized and their right inguinal mammary gland was dissected. The isolated mammary glands were immediately immersed in 10% neutral buffered formalin. Then, it was introduced into tissue embedding cassettes, dehydrated, cleared, and embedded in paraffin wax as per the standard protocol. Five micrometer thick paraffin sections were cut using a rotator microtome (CUT6062, SLEE Medical GmbH, Germany). The tissue sections were then stained for Hematoxylin & Eosin staining for assessment of the microscopic structures of the gland at 450x magnification through Motic microscope (Motic DMB1 Digital Upright Microscope, India).[14]

Behavioral Studies

Behavioral studies of 2nd Generation of the rabbit does were done through following standard behavioral studies models:

Maze test: Number of crossings attempted in the maze

Gait and Movement test: Scores were recorded

Buzzer test: It was performed to observe anxiety effect on the animal due to ringing of a buzzer. The time for which animal was stand still was noted.

Exploratory behavior test: How long animals took to search for feed. [15]

Immunoodulatory study:

Neutrophil adhesion test

Male Swiss albino mice (25–30 g) were divided into four groups, consisting of six animals each. Group I – Control (vehicle 10 ml/kg p.o.), Group II – Standard (Levamisole 25mg/kg), Group III –ATTEB (150 mg/kg p.o.), Group IV – ATTEB (300 mg/kg p.o.). Animals were treated as shown above orally for the 14 days. On the 14thday of drug treatment, blood samples were collected by puncturing the retro-orbital plexus into heparinized vial and analyzed for total leucocyte counts (TLC) and differential leucocyte counts(DLC). After initial counts, blood samples were incubated with 80 mg/ ml of nylon fibers for 15 min. at 37 ° C. The incubated blood samples were again analyzed for TLC and DLC. The product of TLC and percentage

neutrophil gives Neutrophil Index (NI) of blood sample. Percent neutrophil adhesion was calculated as shown below

$$\text{Neutrophil adhesion (\%)} = \frac{NIu - NIt}{NIu} \times 100$$

Where, NIu = Neutrophil index of untreated blood sample.

NI_t= Neutrophil index of treated blood sample.

Carbon clearance test

Male Swiss albino mice (25–30 g) were divided into four groups, consisting of six animals each. Group I – Control (vehicle 10 ml/kg p.o.), Group II – Standard (Levamisole 25mg/kg), Group III –ATTEB (150 mg/kg p.o.), Group IV – ATTEB (300 mg/kg p.o.). Animals were treated as shown above orally for 5 days. At the end of 5 days, carbon ink suspension (0.1 ml) was administered into the tail vein 48 h after the last treatment. At 0 and 15 min, blood samples were taken from the retro-orbital sinus (in 5 ml of EDTA solution). A 25 ml sample was combined with 2 ml of a 0.1% sodium carbonate solution, and its absorbance at 660 nm was measured.

The phagocytic index was calculated using the following equation:

Phagocytic index = k (sample)/ k (control)

Where, K = (Log OD1- Log OD2) /15

OD1 = optical densities at 0 min and,

OD2 = optical densities at 15 min, respectively.

Effervescent Tablet formulation:

For preparation of tablets, following method was followed:

Step I: Add Extract (Shatavari, Methi, Guduchi) with Lactose and mix well. Add Tartaric acid, Sodium bicarbonate, and Sucrose to this mixture and mix.

Step II: Mix 2% PVP in IPA 2%and Sodium benzoate.

Add the mixture obtained from step II to the mixture obtained by step- I drop wise to obtain lumps. Pass these lumps through sieve number 20. The product was dried in a tray for 1 hr. at 60ºC to 70ºC. Pass the dried blend through sieve number 100 to get granules. Mix 1.5% w/w

Talc and Magnesium stearate and mix well for 15 minutes. The dried granules were compressed to get the tablets through tablet compression machine. [19]

Effervescent Tablet evaluation:

Following parameters were assessed: Weight variation, thickness, diameter, hardness, friability, disintegration time as per Indian Pharmacopoeia.[19]

Effervescent granule formulation:

Material: The hydro alcoholic extracts of Shatavari, Guduchi, and Methi were prepared in house and evaluated for parameters specified as per WHO guidelines and found to comply all. Merck India Ltd., Mumbai, India, provided tartaric acid, sodium bicarbonate, lactose, sodium benzoate, and sucrose. Sigma Aldrich in India provided the polyvinyl pyrrolidone (PVP). The study's other ingredients and chemicals were of analytical grade.

For preparation of granules, following method was followed:

Step I: To make binder solution, mix HPMC in IPA 2%and Sucrose.

Step II: Mix Extract (Shatavari, Methi, Guduchi), Citric acid, Tartaric acid, and Sodium bicarbonate.

Add the mixture obtained from step I to the mixture obtained by step- II drop wise to obtain lumps. Pass these lumps through sieve number 60. Tray dry the product for 1 hr. at 60ºC to 70ºC. [18]

Effervescent granule evaluation:

The following evaluation parameters bulk density, tap density, Carr's index, Hausner's ratio, angle of repose and effervescent time were evaluated as per Indian Pharmacopoeia. [18]

Stability Studies

Stability studies were performed as per the ICH guidelines. Specifically, three time points were selected for accelerated stability studies. Tablets were kept at 40ºC ± 2ºC, Relative Humidity 75% ± 5% for 0, 3 and 6 months. Following keeping tablets for selected duration, tablets were evaluated for various parameters including disintegration time, weight variation, thickness, diameter, hardness, friability. Granules were evaluated for various parameters including bulk density, tap density, Carr's index, Hausner's ratio, angle of repose and effervescent time. [2021]

Statistical analysis

Results were expressed as mean ± standard deviation (SD) (n=6), and statistical analysis was performed using Graph Pad Prism 5.0 (San Diego, CA, USA) software, where the value of *p < 0.05, **p<0.01 and ***p<0.001 were considered as indicating a statistical significance. Two-Way Analysis of Variance (ANOVA) followed by Bonferroni post hoc test was used to compare replicated means by row.

Results:

Hydroalcoholic extract of *Asparagus racemosus*, *Tinospora cordifolia* and *Trigonella foenum graceum* were prepared and analysed.

Botanical Name:	*Asparagus racemosus* Dry Extract
Vernacular Name:	Shatavari
Part used:	Root
Solvent:	Hydroalcoholic
Bioactive constituents:	Saponin
Extract yield:	10% of initial drug weight

Table 2 A. Organoleptic Characteristics of *Asparagus racemosus*

	Results	Specification
Color	Off white	White to off white
Odor	Characteristic	Characteristic
Taste	Characteristic	Characteristic
Physical appearance	Hygroscopic powder	Hygroscopic powder

Table 2 B. Physicochemical Characteristicsof *Asparagus racemosus*

	Results	Specification
WSE	89.97%	NLT 70%
ASE 50	82.54%	NLT 70%
PH 1%w/v solution	5.42	3-7

LOD	5.32%	NMT 7%
Ash content	3.05%	NMT 15%
Acid insoluble Ash	Negligible	NMT 2%

Botanical Name: *Tinospora cordifolia* Dry Extract
Vernacular Name: Guduchi
Part used: Stem
Solvent: Hydroalcoholic
Extract yield: 10% of initial drug weight

Table 3 A. Organoleptic Characteristics of *Tinosporacordifolia*

	Results	Specification
Color	Brown	Brown
Odor	Characteristic	Characteristic
Taste	Bitter	Bitter
Physical appearance	Hygroscopic powder	Hygroscopic powder

Table 3B. Physicochemical Characteristicsof *Tinosporacordifolia*

	Results	Specification
WSE	87.54%	NLT 70%
ASE 50	85.71%	NLT 70%
PH 1%w/v solution	5.82	3-7
LOD	1.63%	NMT 7%
Ash content	4.28%	NMT 15%
Acid insoluble Ash	0.37%	NMT 2%

Botanical Name: *Trigonella foenum graceum* Dry Extract

Vernacular Name: Methi

Part used: Seed

Solvent: Hydroalcoholic

Extract yield: 12.5% of initial drug weight

Table 4 A. Organoleptic Characteristics of *Trigonellafoenumgraceum*

	Results	Specification
Color	Brown	Brown to Dark brown
Odor	Characteristic	Characteristic
Taste	Characteristic	Characteristic
Physical appearance	Hygroscopic powder	Hygroscopic powder

Table 4 B. Physicochemical Characteristicsof *Trigonellafoenumgraceum*

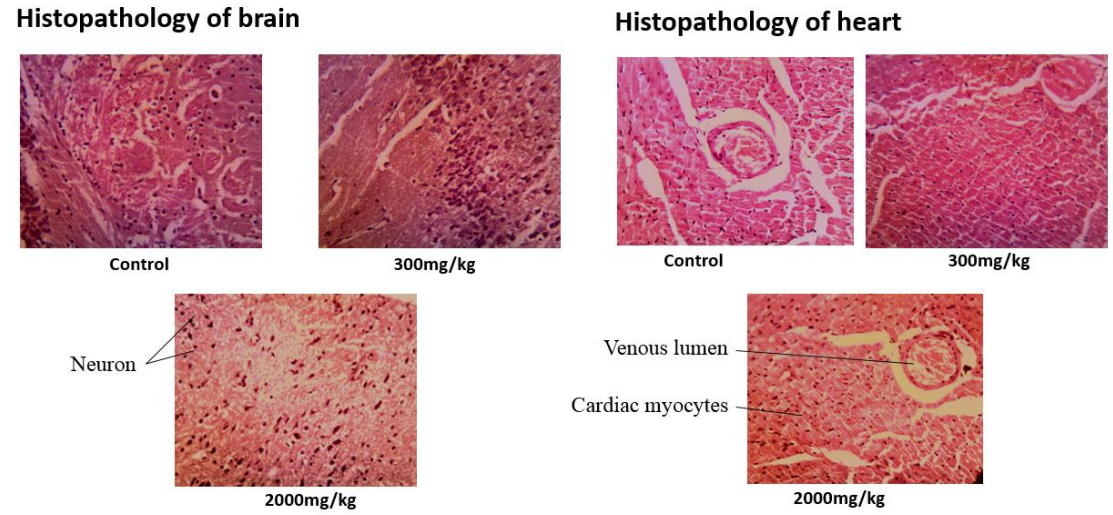
	Results	Specification
WSE	88.29%	NLT 70%
ASE 50	79.3%	NLT 70%
PH 1%w/v solution	6	3-7
LOD	3.84%	NMT 7%
Ash content	0.45%	NMT 15%
Acid insoluble Ash	Negligible	NMT 2%
Saponin	21.38%	NLT20%

Toxicity Studies

1. **Acute Toxicity Studies (14 days, as per OECD Guidelines Test Guideline 423)**

After drug administration for 14 days, it was observed that the body weight of animals has no significant change among groups under study. Food and water intake of animals was also not significantly altered. Histopathological analysis of the vital organs was done which is found to be normal.

A



B

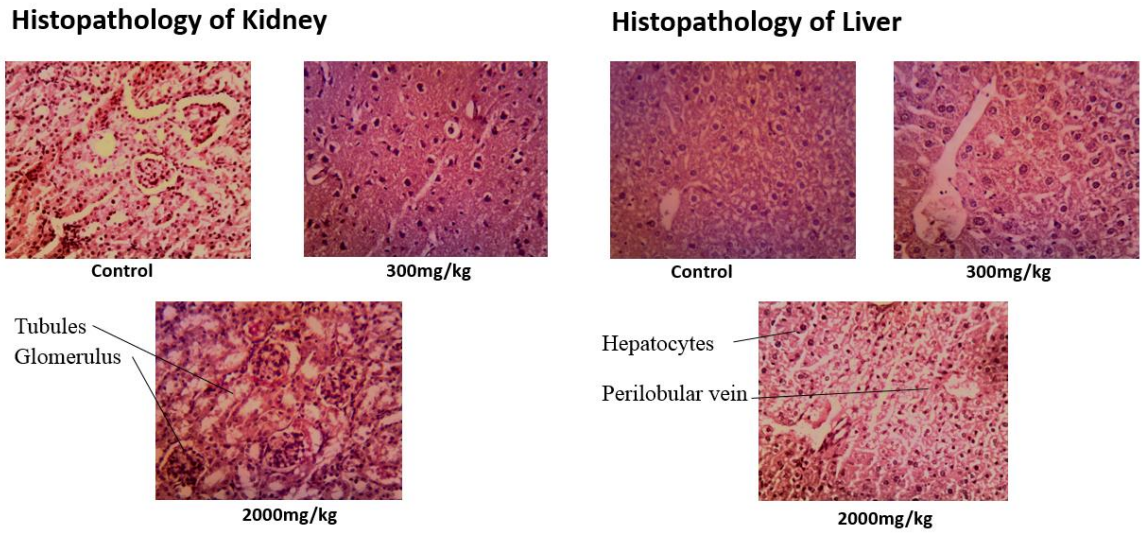


Figure 3. Histopathological analysis of the vital organs, A. Brain, Heart, B. Kidney, Liver.

C

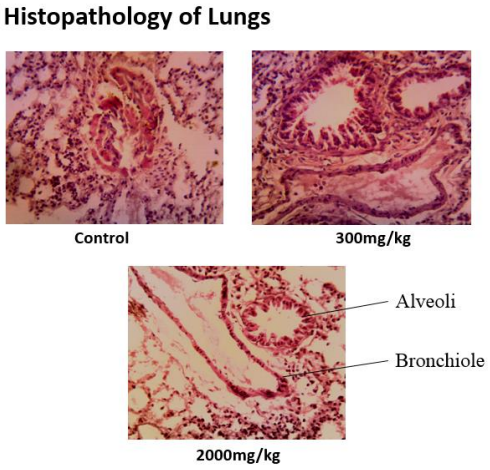
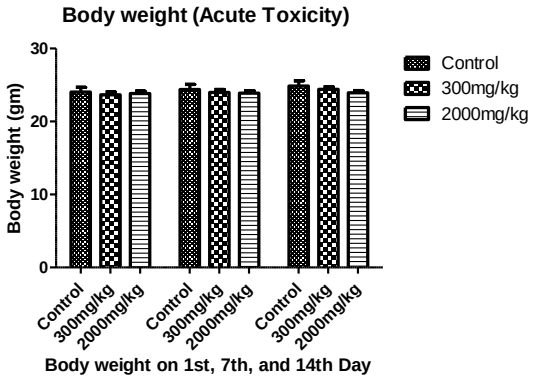


Figure 3. Histopathological analysis of the vital organs, C. Lungs.

A



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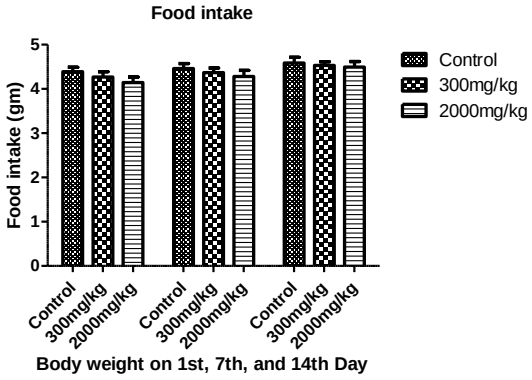

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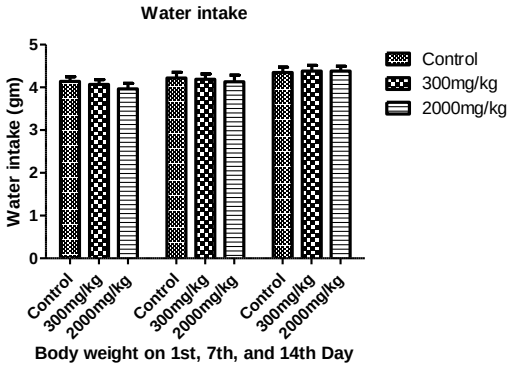


Figure 4. A. Body weight of mice, B. Food intake and C. Water intake of mice. Value expressed as Mean ± STD. Data are considered statistically significant when $p < 0.05$.

Table 5. Cage side observations for 14 days of control and treated mice at a single dose of 5000mg/kg BW

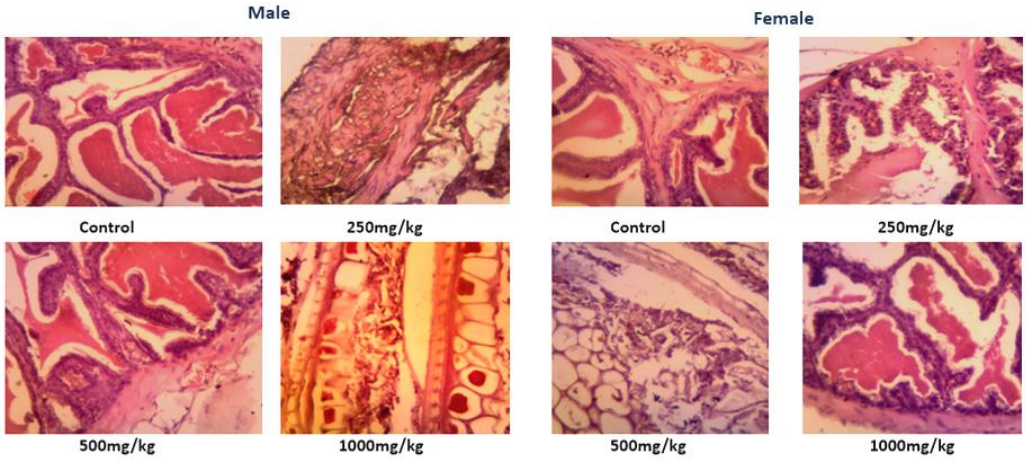
Parameters	Observations								
	Group 1			Group 2			Group 3		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Condition of fur	N	N	N	N	N	N	N	N	N
Change in skin	NC	NC	NC	NC	NC	NC	NC	NC	NC
Body posture	N	N	N	N	N	N	N	N	N
Eye colour	NC	NC	NC	NC	NC	NC	NC	NC	NC
Diarrhoea	A	A	A	A	A	A	A	A	A
Respiration	N	N	N	N	N	N	N	N	N
Sedation	A	A	A	A	A	A	A	A	A
Drowsiness	NP	NP	NP	NP	NP	NP	NP	NP	NP
Coma	A	A	A	A	A	A	A	A	A
Mortality	Al	Al	Al	Al	Al	Al	Al	Al	Al

N- Normal, NC- No Change, A- Absent, NP- Not Present, Al-Alive.

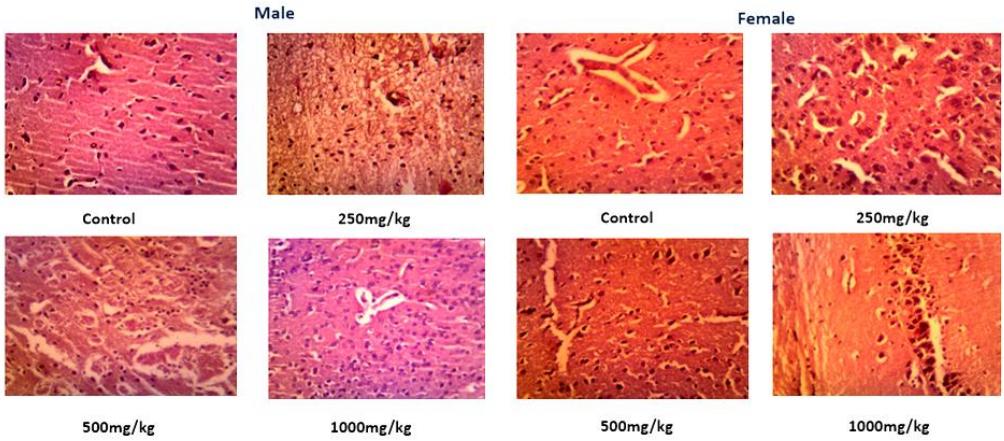
2. Repeat Dose Toxicity Studies (28 days, as per OECD Guidelines Test Guideline 407)

Body weight and organ weight: The 28 days repeated administration of drug mixture in mice showed no significant changes in body weight of both male and female mice when compared with respective control group. Also, the weight of different organs was also having insignificant difference as compared to control group. The microscopic examination of all the organs of mice too revealed no change compared to control.

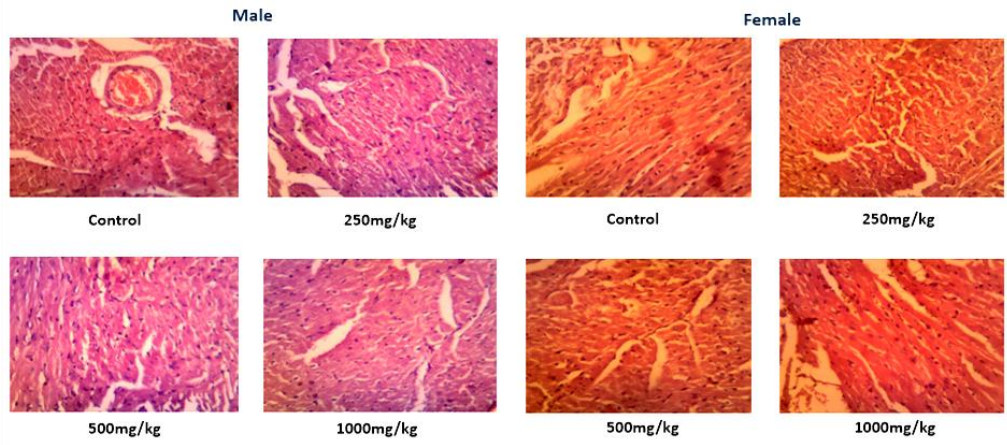
A. Histopathology of Adrenal glands



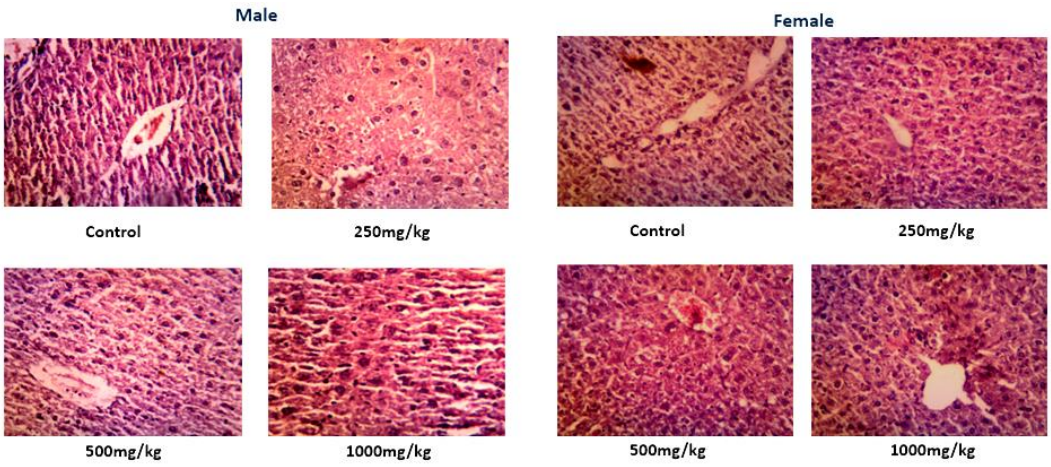
B. Histopathology of Brain



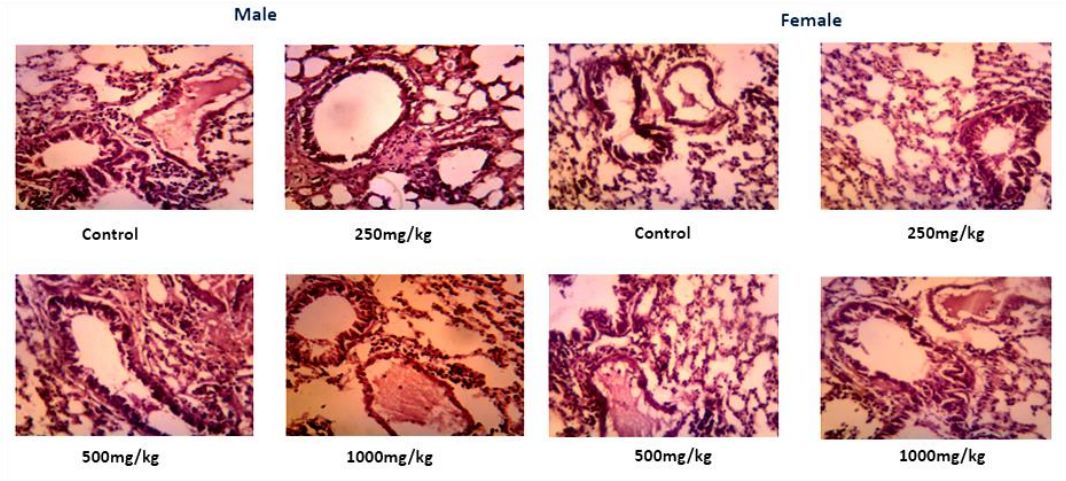
C. Histopathology of Heart



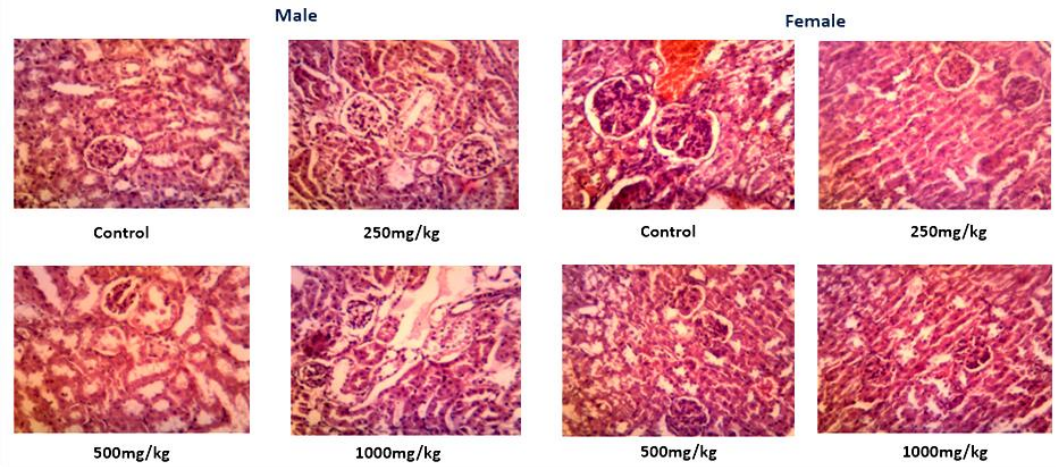
D. Histopathology of Liver



E. Histopathology of Lung



F. Histopathology of Kidney



G. Histopathology of Spleen

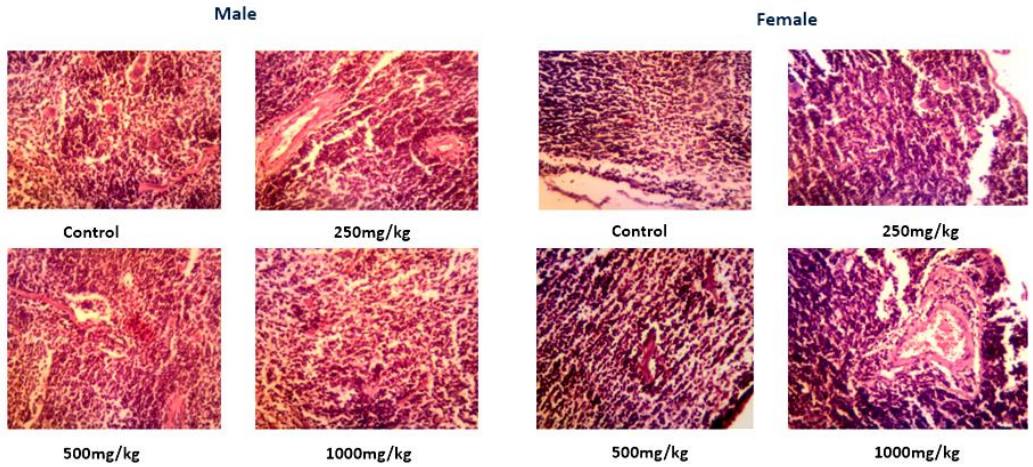
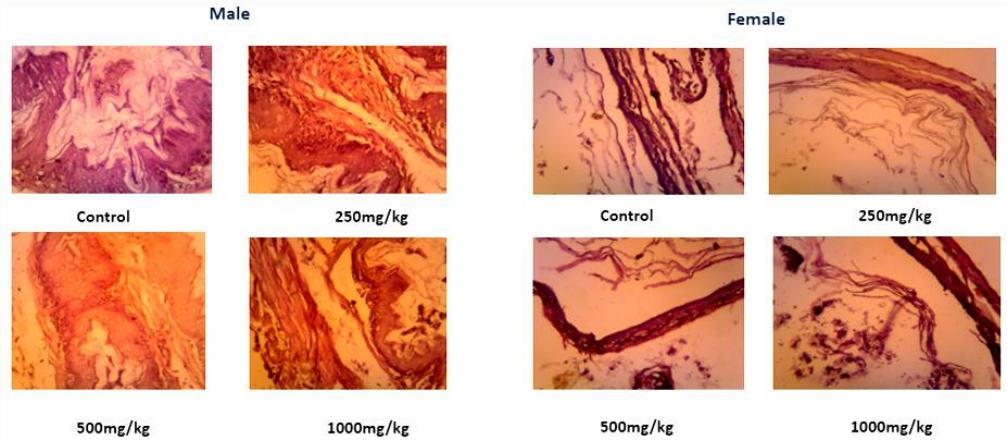


Figure 5. Histopathological analysis of the vital organs of Male and Female mice, E. Lungs, F. Kidney. G. Histopathology of Spleen

H. Histopathology of Stomach



I. Histopathology of Testis and ovaries

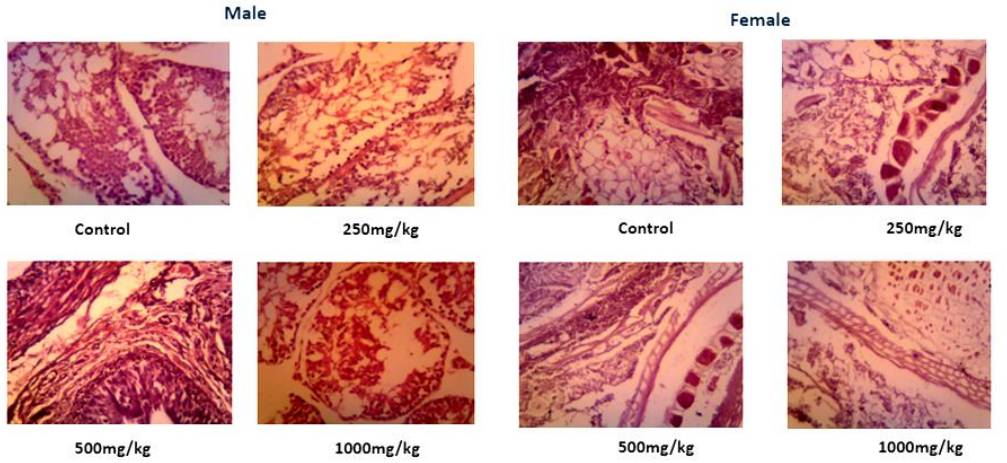


Figure 5. Histopathological analysis of the vital organs of Male and Female mice, H. Stomach, I. Testis and Ovaries.

Male Female:

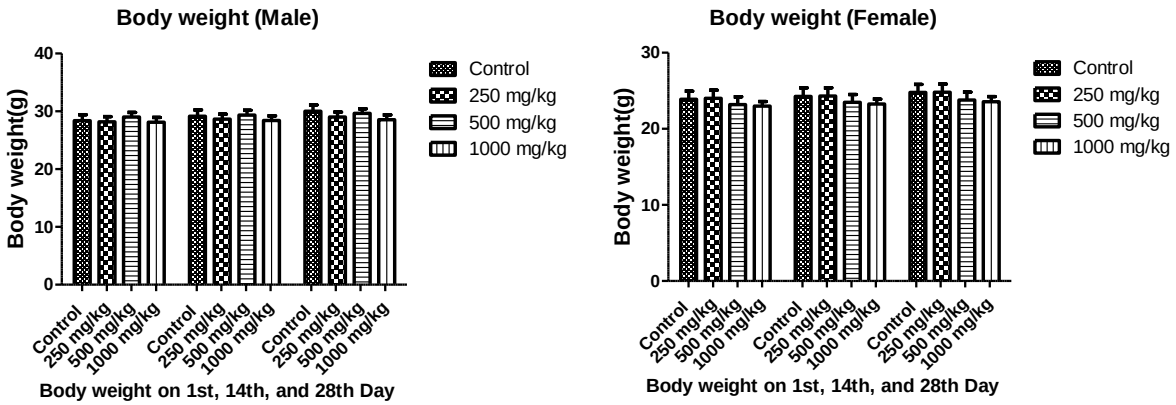


Figure 6. Body Weight of Male and Female mice at time interval of 14 days. Value expressed as Mean ± STD. Data are considered statistically significant when $p < 0.05$.

For the necroscopic study, various organs of the rat were isolated and weighed, and histopathological studies were performed.

Table 6. Organ weight of animals' post ATTEB treatment for 28 days

	Male				**Female**			
	Group I	**Group II**	**Group III**	**Group IV**	**Group I**	**Group II**	**Group III**	**Group IV**
Body Weight (g)	30.01± 1.12	29.02± 0.87	29.65± 0.81	28.56± 0.805	24.79± 1.05	24.818 ±1.07	23.792 ±1.04	23.552 ±0.68
Liver (g)	1.624± 0.09	1.532± 0.06	1.674± 0.07	1.68±0. 12	1.43±0. 05	1.386± 0.043	1.362± 0.037	1.406± 0.052
Heart (g)	0.1984 ±0.007	0.206± 0.009	0.2024 ±0.012	0.2008 ±0.004	0.1582 ±0.006	0.1612 ±0.004	0.1556 ±0.004	0.15±0. 006
Spleen (g)	0.2058 ±0.008	0.1974 ±0.009	0.2078 ±0.01	0.2174 ±0.007	0.1708 ±0.003	0.174± 0.008	0.164± 0.006	0.1616 ±0.007
Pancreas (g)	0.1126 ±0.012	0.1202 ±0.006	0.1214 ±0.007	0.1108 ±0.008	0.1248 ±0.006	0.12±0. 006	0.1214 ±0.005	0.1252 ±0.004
Lung (g)	0.224± 0.024	0.232± 0.0164	0.252± 0.0178	0.224± 0.011	0.183± 0.007	0.1776 ±0.005	0.1748 ±0.005	0.1792 ±0.004
Testes/ ovary (g)	0.228± 0.026	0.24±0. 012	0.234± 0.021	0.254± 0.017	0.131± 0.002	0.132± 0.004	0.1282 ±0.001	0.1312 ±0.003
Brain (g)	0.432± 0.038	0.452± 0.019	0.43±0. 019	0.46±0. 025	0.4164 ±0.03	0.4082 ±0.025	0.3972 ±0.022	0.4076 ±0.012
Kidney (g)	0.45±0. 025	0.47±0. 027	0.458± 0.022	0.45±0. 023	0.3562 ±0.01	0.3666 ±0.007	0.3634 ±0.006	0.3444 ±0.006
Stomach (g)	0.2312 ±0.009	0.2214 ±0.009	0.221± 0.01	0.2234 ±0.007	0.1912 ±0.01	0.1936 ±0.01	0.1864 ±0.008	0.1764 ±0.007

The blood and urine from the animal were collected for complete blood count and biochemical analysis of the fluids. Following are the outcome of the complete blood count and biochemical analysis of blood and biochemical analysis of urine.

Table 7. Hematological Parameters A. Female, B. Male

	Male				**Female**			
Parameters	**Group I**	**Group II**	**Group III**	**Group IV**	**Group I**	**Group II**	**Group III**	**Group IV**
Hb (g/dl)	14.152± 0.196	14.356± 0.21	14.166± 0.15	13.982 ±0.16	13.326 ±0.34	13.572 ±0.31	13.672 ±0.29	13.488 ±0.17
RBC (*10^6/µl)	10.954± 0.21	10.366± 0.056	10.296± 0.093	10.21± 0.048	9.474± 0.49	9.81±0. 45	9.624± 0.31	9.582± 0.26
WBC (*10^3/µl)	8.85± 0.4	8.746± 0.33	8.93± 0.118	9.014± 0.214	7.44±1. 18	7.634± 0.96	7.816± 1.18	8.326± 0.92
Platelets (*10^3/µl)	489.8± 20.51	516.4± 19.82	472.6± 21.38	508± 17.56	458.4± 15.49	478.8± 12.39	446.6± 12.93	467± 15.62
Basophils (%)	0.26± 0.03	0.246± 0.02	0.266± 0.02	0.252± 0.02	0.224± 0.06	0.216± 0.06	0.218± 0.07	0.214± 0.05
Lymphocytes (%)	74.932± 4.305	74.414± 4.1	78.67± 4.87	81.582 ±3.89	71.894 ±2.95	73.81± 2.64	76.566 ±2.75	79.462 ±2.14
Monocytes (%)	1.128± 0.12	1.178± 0.06	1.19± 0.05	1.15± 0.1	1.03± 0.07	1.06± 0.06	1.056± 0.05	1.04± 0.04
MCV (fl)	46.292± 2.35	48.192± 2.66	49.796± 2.59	49.964 ±2.74	42.222 ±1.63	44.216 ±1.13	44.974 ±2.16	45.184 ±2.12
MCHC (%)	35.392± 1.11	36.156± 1.42	36.692± 1.08	35.728 ±1.76	33.258 ±0.69	33.772 ±0.31	33.828 ±0.26	32.788 ±0.33
MCH (pg)	14.478± 0.386	14.612± 0.25	14.868± 0.18	14.63± 0.15	14.538 ±0.56	14.826 ±0.51	15.036 ±0.42	14.868 ±0.21
Eosinophils (%)	2.352± 0.087	2.316± 0.04	2.376± 0.05	2.342± 0.04	2.386± 0.16	2.332± 0.11	2.394± 0.13	2.404± 0.10
Neutrophils (%)	23.61± 1.55	21.76± 1.22	22.51± 1.19	23.386 ±1.09	21.63± 2.06	20.97± 0.98	21.74± 0.99	22.288 ±1.00

Table 8. Biochemical analysis of animals: Complete Blood Count, Liver and Kidney function, Serum Electrolyte: A. Female, B. Male

Table 5.8 Complete Blood Count, Liver function, Kidney function, and Serum Electrolyte

	Male	**Female**

Parameters	Group I	Group II	Group III	Group IV	Group I	Group II	Group III	Group IV
BUN (mg/dl)	25.15± 1.11	26.388 ±1.38	24.198 ±0.90	24.798 ±0.83	21.612 ±2.39	23.374 ±2.83	22.388 ±1.28	22.916 ±0.76
Creatinine (mg/dl)	0.428± 0.05	0.472± 0.06	0.522± 0.07	0.514± 0.05	0.448± 0.05	0.436± 0.02	0.458± 0.02	0.486± 0.03
Glucose (mg/dl)	113.53 4±5.06	115.89 2±5.29	109.76 8±3.97	105.46 6±1.53	114.64 2±5.22	112.44 4±5.15	108.94 2±2.58	104.32 8±4.77
Total protein (g/dl)	5.832± 0.73	6.18±0. 56	5.512± 0.47	4.986± 0.34	5.562± 0.31	5.982± 0.39	5.292± 0.28	4.99±0 .204
ALT (SGPT) (U/L)	42.634 ±2.56	41.574 ±1.77	41.382 ±2.29	42.038 ±2.19	39.61± 2.74	38.13± 1.86	37.594 ±0.63	37.814 ±0.63
AST (SGOT) (U/L)	121.13 2±2.16	118.58 ±2.41	120.79 4±2.14	120.11 2±1.45	116.29 6±2.30	114.22 8±0.95	119.58 6±1.20	120.04 6±2.85
ALP (U/L)	252.35 6±12.0 6	263.73 2±11.9 2	254.18 2±13.5 0	258.77 8±15.1 6	276.15 6±8.91	280.31 ±6.15	283.86 8±10.1 3	286.94 8±8.62
Total bilirubin (mg/dl)	0.358± 0.03	0.29±0. 04	0.322± 0.05	0.39±0. 04	0.386± 0.018	0.368± 0.032	0.358± 0.016	0.406± 0.02
Total cholesterol (mg/dl)	97.414 ±4.52	96.34± 2.85	94.37± 3.55	92.27± 3.64	98.44± 3.96	97.478 ±4.13	94.218 ±2.56	93.124 ±2.98
Triglyceride (mg/dl)	58.566 ±2.61	60.368 ±3.07	57.38± 3.53	55.912 ±3.27	60.608 ±2.54	62.568 ±2.88	61.938 ±2.24	59.55± 2.01
Sodium (mEq/L)	138.23 8±6.72	131.73 8±5.17	135.56 2±4.29	129.54 2±3.48	130.82 4±3.43	129.59 ±1.27	128.75 4±2.82	127.48 ±3.19
potassium (mEq/L)	3.966± 0.23	4.13±0. 19	4.056± 0.15	3.824± 0.09	4.072± 0.18	4.212± 0.1	4.17±0. 09	3.984± 0.09
chloride (mEq/L)	103.70 4±5.16	102.63 ±3.17	101.46 ±2.85	98.596 ±2.69	101.25 8±1.84	100.23 8±2.18	98.768 ±1.14	98.412 ±1.80

Haematological and Biochemical parameters:

The effect of drug administration on haematological and biochemical parameters after repeated dosing for 28 days is given in Table 9. No significant changes were observed in haematological parameters of treated animals when compared to control group of animals. The biochemical parameters of the treated animals also remained within limit.

The electrolyte level in blood serum showed no major alteration compared to control group.

The urinary profile of treated animals was also compared to that of control animals. These changes were also insignificant. Glucose and protein remained absent in all groups of mice.

Results: Results of acute and repeat dose toxicity indicated the blend of extracts is not having any noticeable toxicity in mice.

Biological studies:

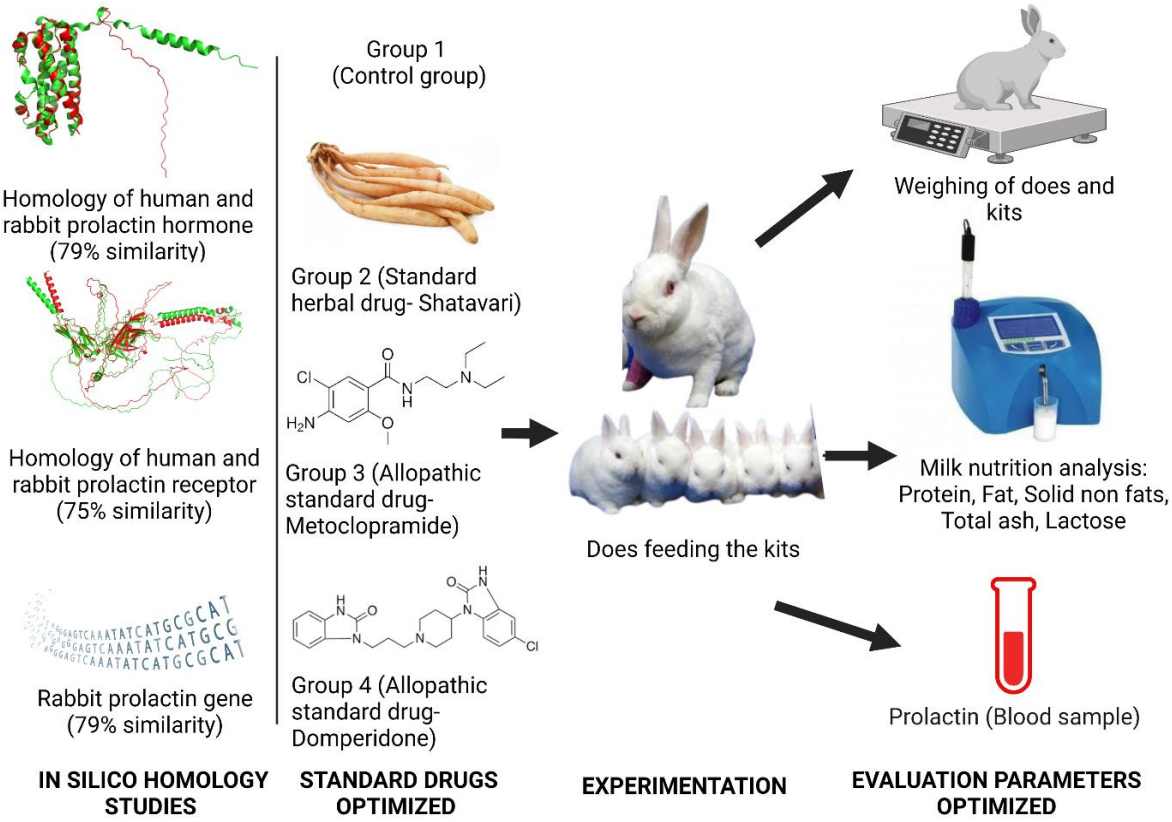

Homology of human and rabbit prolactin hormone (79% similarity)
Homology of human and rabbit prolactin receptor (75% similarity)
Rabbit prolactin gene (79% similarity)
IN SILICO HOMOLOGY STUDIES
Group 1 (Control group)
Group 2 (Standard herbal drug- Shatavari)
Group 3 (Allopathic standard drug- Metoclopramide)
Group 4 (Allopathic standard drug- Domperidone)
STANDARD DRUGS OPTIMIZED
EXPERIMENTATION
Does feeding the kits
Weighing of does and kits
Milk nutrition analysis: Protein, Fat, Solid non fats, Total ash, Lactose
Prolactin (Blood sample)
EVALUATION PARAMETERS OPTIMIZED

Figure 2. Graphical Abstract of Lactation study

Efficacy studies:

Experimental Animal: Rabbit (lactating female, behavioral studies and body weight data were collected from the kits of respective does)**.**

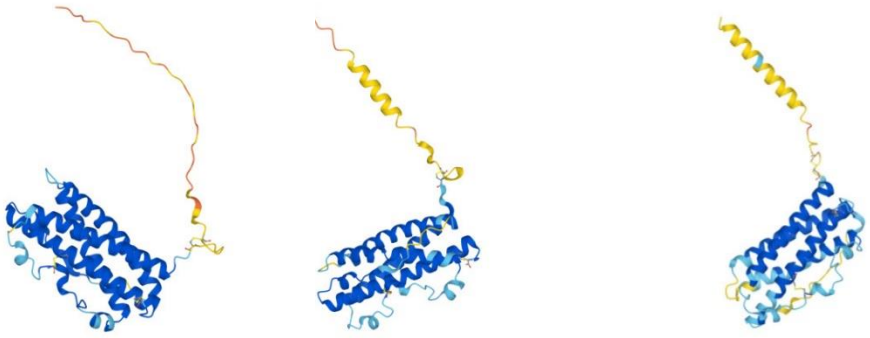
Treatment: Once in a day for 21 days.

Drug: Individual and Blend of three extracts in 1:1:1 proportion (Shatavari, Methi, Guduchi)

Human, Rabbit, and Rat Prolactin (Prl) gene, hormone, and their receptors homology study

Lactation is primarily modulated by secretion of hormone Prl. The pathway consists of Prl gene expression, hormone release and binding with Prl receptors. To test the hypothesis that rabbits

have any similarity in the lactogenic system of humans, a homology study was performed in-silico. In the scientific research papers, the most frequently employed animal model is Rat, thus rat was also considered for relative comparison for the same.



Human Prolactin Rabbit prolactin Rat prolactin

Figure 8. Homology of rabbit prolactin hormone with human

A comparison through sequence alignment of prl gene, prl hormone and prl receptor was established as follows.

A. **B.**

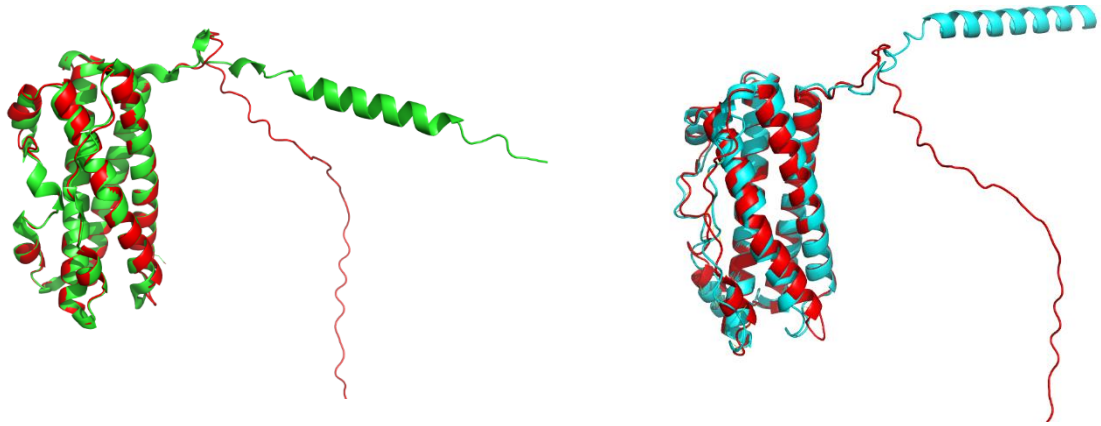


Figure 9. Alignment of human prolactin (Red) with rabbit (green) and rat (cyan).

A. Superimposed Prl Hormone of human and rabbit: Identities: 179/227 (79%), Positives: 204/227 (89%), Gaps: 0/227 (0%).

B. Superimposed Prl Hormoneof human and rat: Identities: 128/206 (62%), Positives: 160/206 (77%), Gaps: 2/206 (0%).

Prolactin Gene

The similarities of human and rabbit Prl gene sequences were deduced by aligning them through BLASTN, which exhibited a 71% gene sequence similarity with a query coverage of 79%. In

contrast, the human and rat Prl gene sequences were found to be 73% similar, but with only 13% of query coverage.

Prolactin Hormones

To explore the similarity in AA (Amino Acid) sequences of human, rabbit, and rat Prl hormones, the respective sequences were downloaded from Uniprot and were aligned using BLASTP. The sequence similarities between human and rabbit Prl were found to be 79% with 204 positive alignments out of 227, while between human and rat the alignment was 62% with 160 positive alignments out of 206.

A. **B.**

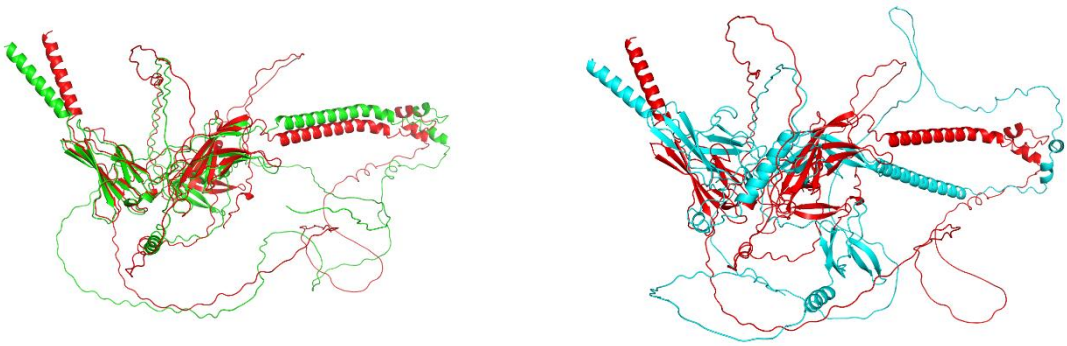


Figure 10. Alignment of human Prl receptor (Red) with rabbit (green) and rat (cyan).

A. Superimposed Prl receptor of human and rabbit: Identities: 470/624 (75%), Positives: 519/624 (83%), Gaps: 10/624 (1%)

B. Superimposed Prl receptor of human and rat: Identities: 422/618 (68%), Positives: 490/618 (79%), Gaps: 12/618 (1%)

Since a high sequence alignment cannot guarantee a high structural alignment, it was thought worthwhile to superimpose the 3D structures of human-rabbit and human-rat Prl respectively. We searched the protein data bank (PDB) for this purpose and were able to find human Prl structure, but experimental structures of rat and rabbit Prl were not available. Subsequently, a search of Computed Structure Models (CSM) in the PDB resulted in finding the AlfaFold structures of Prl hormone for all the three species. We then superimposed the AlfaFold generated human Prl CSM on the rabbit Prl CSM and rat Prl CSM respectively. The results of superimposition showed a high structural similarity between both the groups.

Prolactin receptor

The Prl gene and hormone of rabbit showed significant analogy in sequence alignment study which is high as compared to sequence analogy of rat prl gene and hormone with that of human. Finally, hormones have to bind with prl receptor to exert its actions. Thus, prl receptor of human was aligned with rabbit and rat prl receptor. Superimposed prl receptor of human with rabbit have 75% identities (470/624) with 83% positives (519/624) while superimposed human prl receptor with that of rat have 68% identities (422/618) with 79% positives (490/618).

Milk Nutrition Analysis

Following graphical representation depicted positive response of the extracts on total milk production in 21 days and milk production comparison of each day and analysis of the Lactose, Solid Non-Fat, Fats, and Protein present in milk.

A comparative analysis of the over all nutrion level of each group of milk with individual component of nutrition is given which clearly indicating the superiority of the results of treatment group over the control group. There is increase in mother's body weight with treatment.

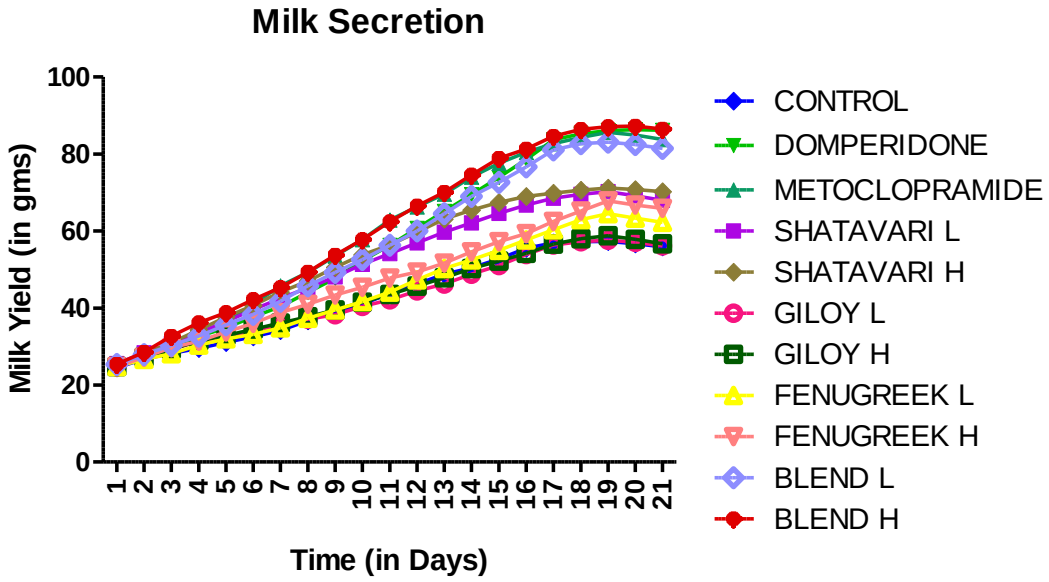


Figure 11. Milk secretion study in various groups of rabbit does for 21 days. Value expressed as Mean ± STD. Data are considered statistically significant when $p < 0.05$.

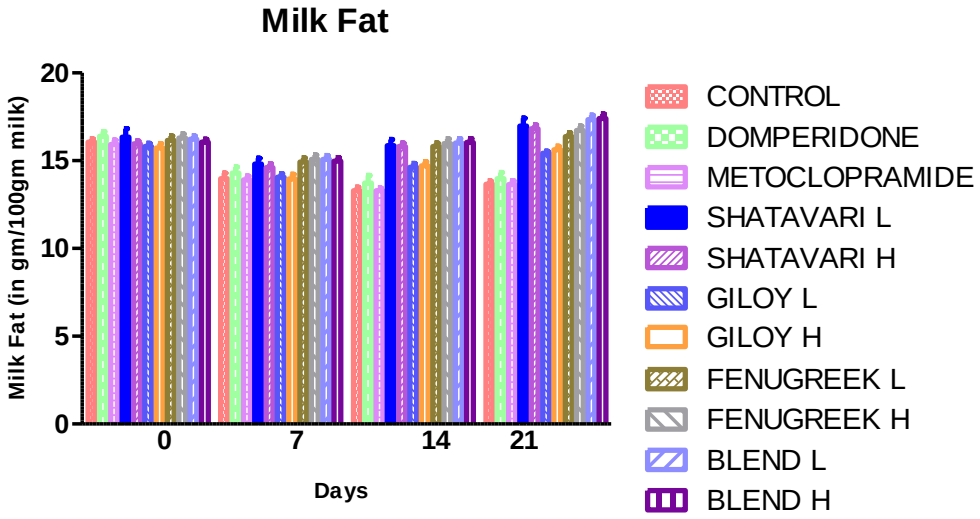


Figure 12. Milk Fat analysis in milk of various groups of rabbit does for 21 days. Value expressed as Mean ± STD. Data are considered statistically significant when $p < 0.05$.

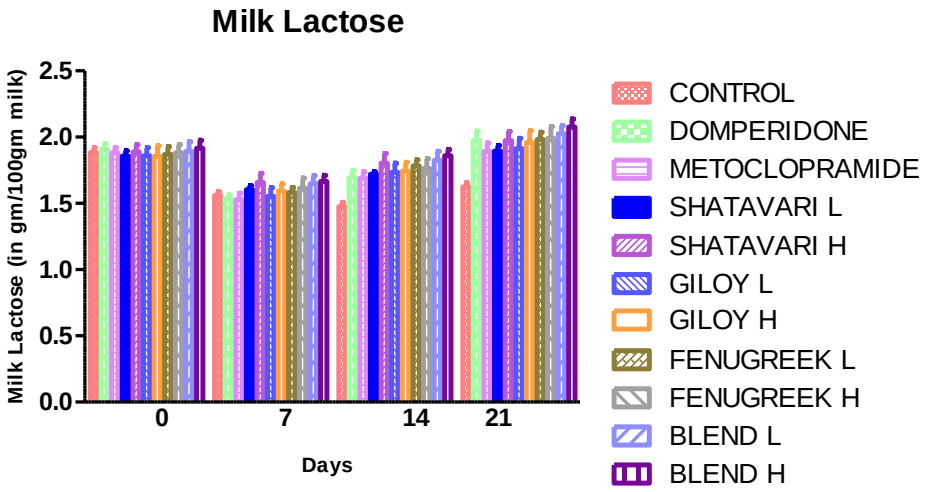


Figure 13. Milk Lactose analysis in milk of various groups of rabbit does for 21 days. Value expressed as Mean ± STD. Data are considered statistically significant when $p < 0.05$.

Results indicated the presence of highest ash value in the higher dose of the blend indicated the increase in mineral content of the milk. The detailed mineral analysis was performed and that confirmed the hypothesis. The Immunoglobulin level in rabbit milk was analysed and found to have higher level in highest treatment group compared to the standard drug treatment group. Similarly, significant higher level of prolactin was observed in the serum of the rabbit

does treated with the high dose of blend indicated that the increase in the milk synthesis in does is due to enhancement of serum prolactin level.

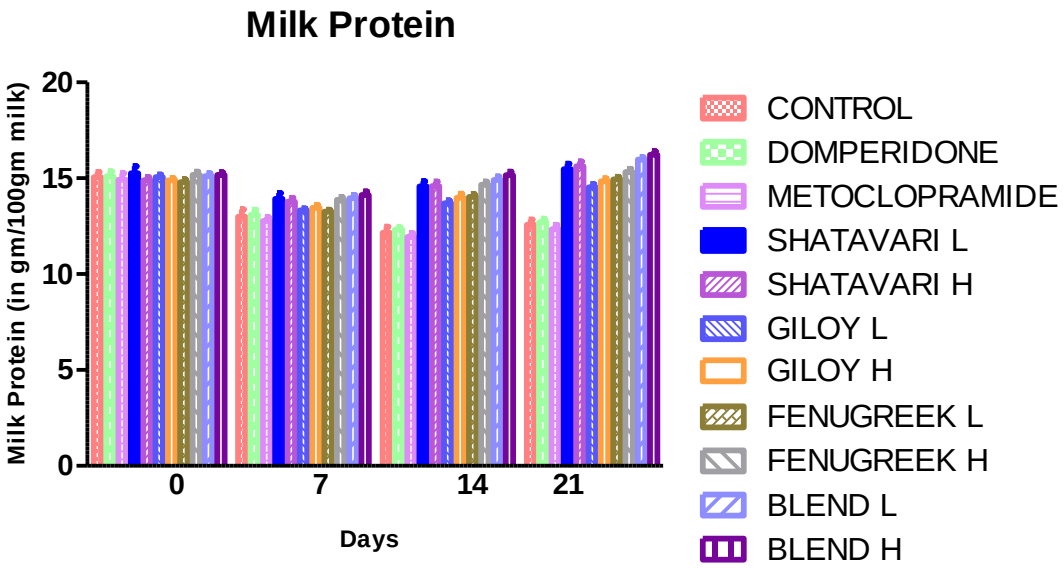


Figure 14. Milk Protein analysis in milk of various groups of rabbit does for 21 days. Value expressed as Mean ± STD. Data are considered statistically significant when $p < 0.05$.

All the results were comparable to standard drug group. Immunoglobulin level was normal in standard drug group compared to higher in highest dose of treatment group.

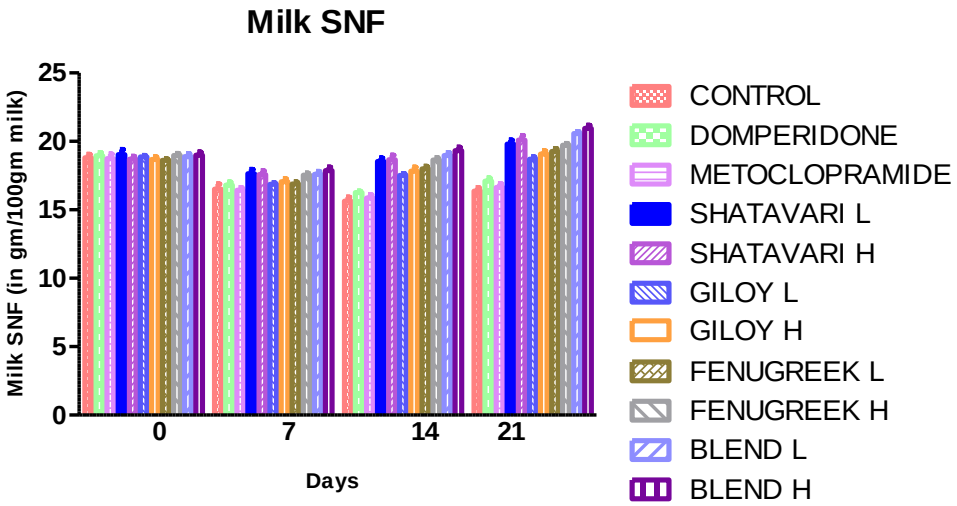


Figure 15. Milk Soli-Non-Fat analysis in milk of various groups of rabbit does for 21 days. Value expressed as Mean ± STD. Data are considered statistically significant when $p < 0.05$.

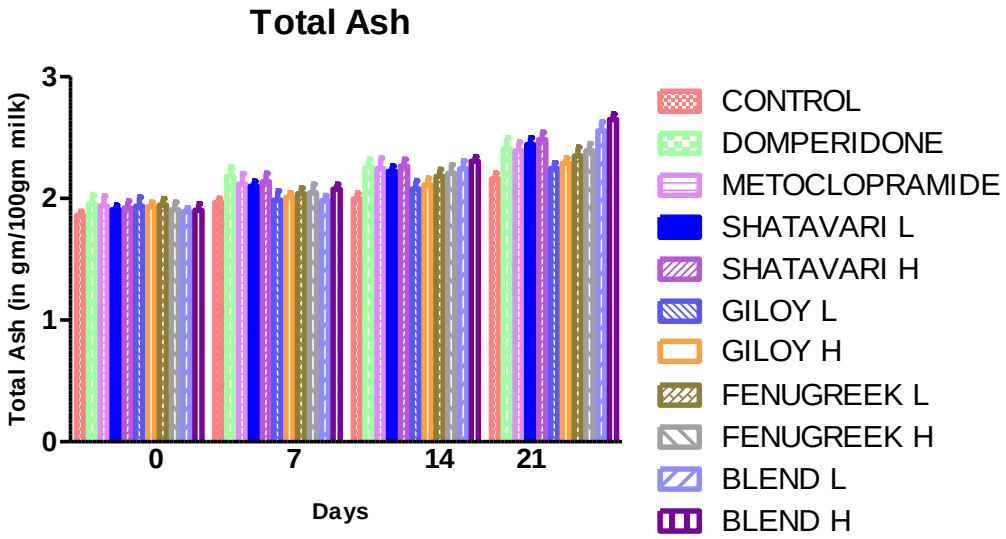


Figure 16. Milk Total Ash analysis in milk of various groups of rabbit does for 21 days. Value expressed as Mean ± STD. Data are considered statistically significant when $p< 0.05$.

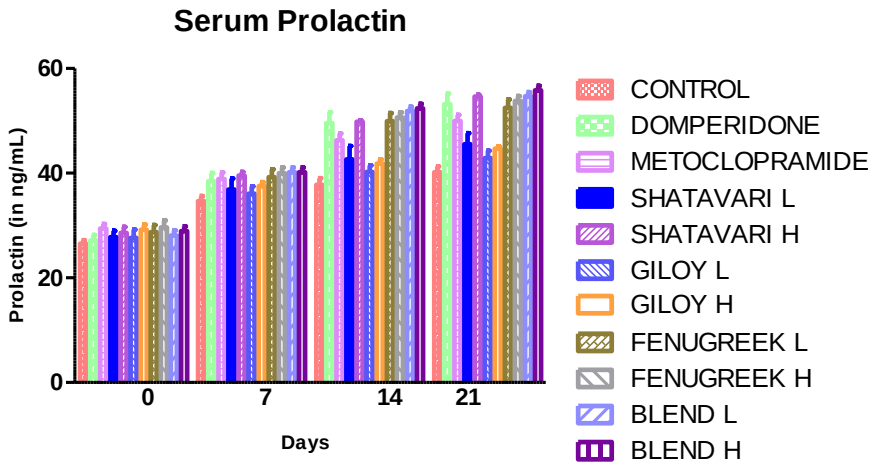


Figure 17. Serum Prolactin analysis in milk of various groups of rabbit does for 21 days. Value expressed as Mean ± STD. Data are considered statistically significant when $p< 0.05$.

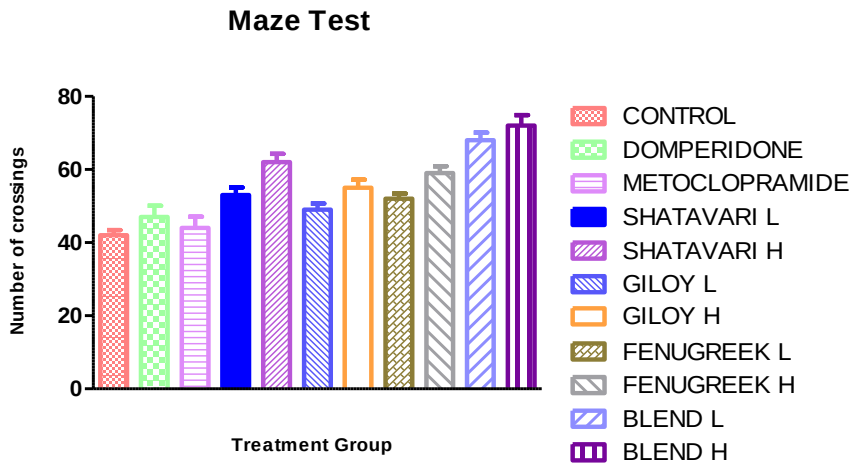
Behavioral Studies

2nd Generation

- **Maze test: Number of crossings attempted in the maze**
- **Exploratory behavior test: How long animal took to search feed**

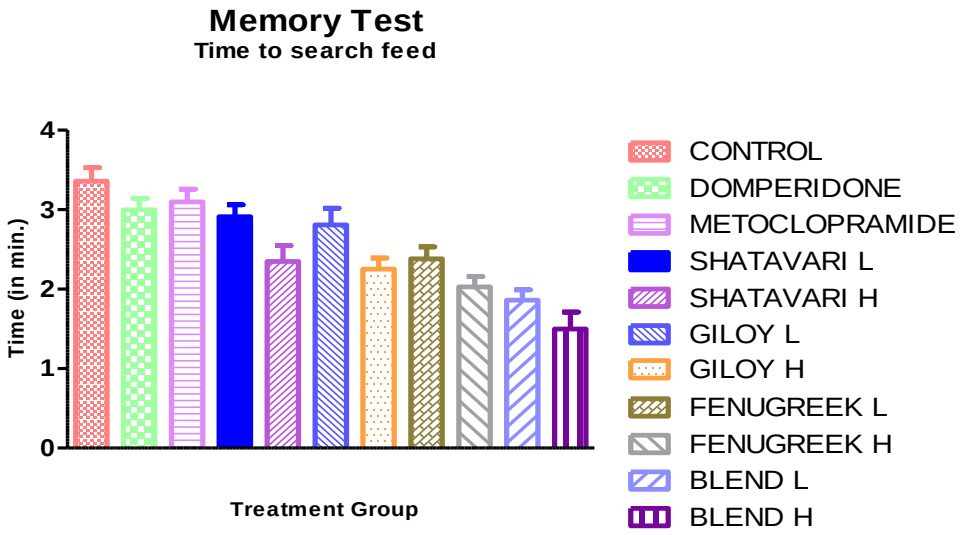
With the hypothesis of having improvement in body and brain development the cognitive and memory skills were tested.

A



The mental development of the kits was analyzed by Maze test, Buzzer test, Memory test and Gait. It was observed in a memory test comprised of determination of time to search feed in a maze. A significant low time was observed with the treatment group of blended high dose D2. The animal's activity in maze was observed for anxiety analysis which had more crossings and standing activity. The results were in consent with earlier results of D2 as highest number of crossing and standing activity. The buzzer induced anxiety was also observed and revealed that the D2 has lowest time of immobility on buzzering compared to untreated and other group.

B



C

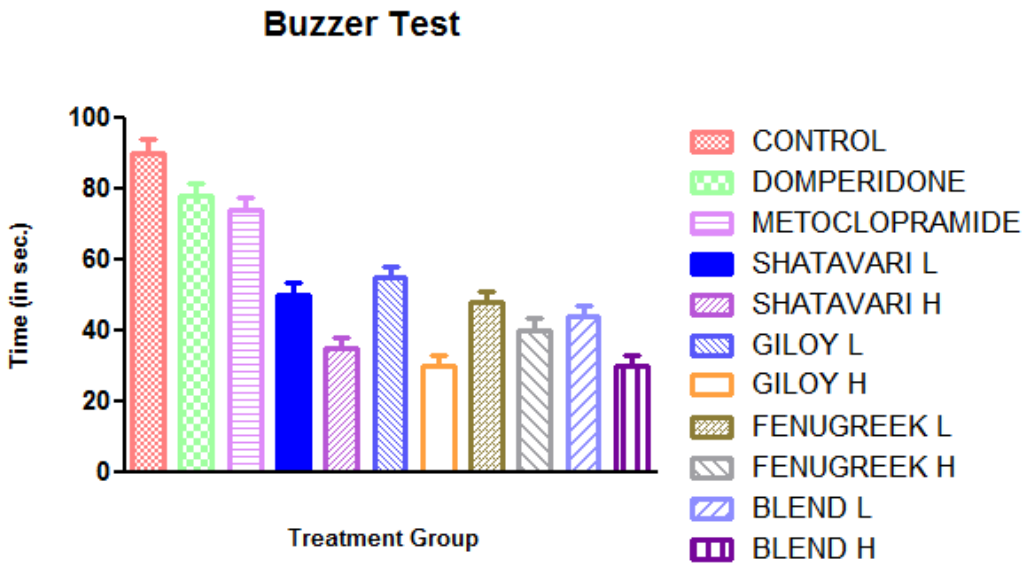


Figure 18. Cognitive analysis of kits: A. Maze Test, B. Memory Test, C. Buzzer Test (induced anxiety time). Value expressed as Mean ± STD. Data are considered statistically significant when $p < 0.05$.

Table 9. Cognitive/behavioral analysis of kits: Gait and Movement scores (For 1 minute, in quit condition):

S.No.	Group	Gait Score (On scale of ++++)	Movement Score (Frolic, on scale of ++++)
1.	E	+++	++
2.	A1	+++	++
3.	A2	+++	+++
4.	B1	+++	+++
5.	B2	++++	+++
6.	C1	+++	++
7.	C2	+++	+++
8.	D1	+++	+++
9.	D2	++++	++++

Gait: +- Non-coordinated, falling like movements, ++- Movements in wavy path, non-coordinated, +++- Movements with more inclination to either side, ++++- Normal balanced movements.

Movement Score: +- No movement, only slight displacement ++- Occasional movement, change in place, +++- Continuous movement with short pause, ++++- Enthusiastic exploratory continuous movement.

Immunomodulatry Studies:

Neutrophil adhesion test: The ATTEB treated groups shown to have higher neutrophil adhesion rates than the control group. ATTEB treatment showed a substantial increase in neutrophil adhesion, pointing to a potential immunostimulant effect of ATTEB

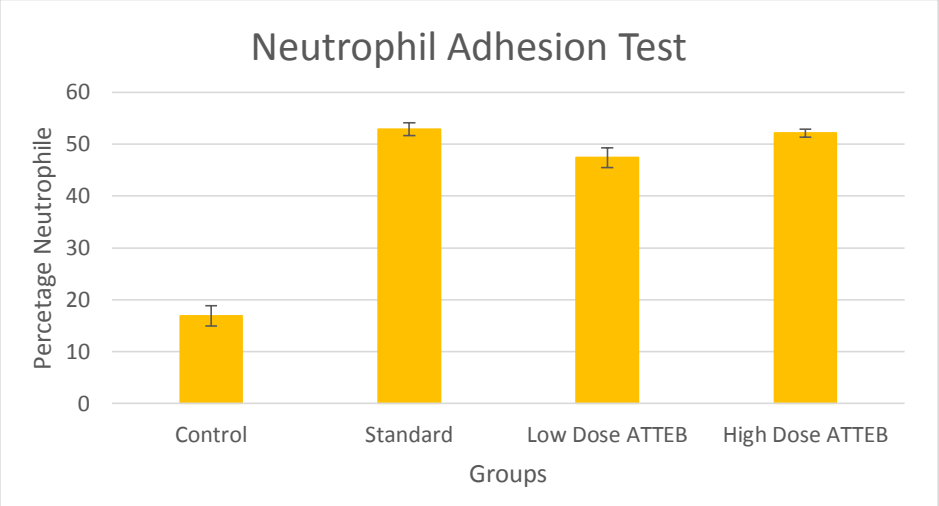


Figure 19. Neutrophil Adhesion Test of control and treated groups. Value expressed as Mean ± STD. Data are considered statistically significant when $p < 0.05$.

Table 10 Neutrophil Adhesion Test

	TLC (103/mm3) A		Neutrophil (%) B		Neutrophil index A*B		Neutrophil adhesion (%)
	UB	NFTB	UB	NFTB	UB	NFTB	
Control	6.095± 0.28	5.6±0. 32	27.86± 1.23	25.19± 1.33	170.07± 15.35	141.52± 15.65	16.91±1.94
Standard	9.865± 0.27	8.58± 0.33	32.01± 0.92	17.35± 0.68	315.97± 17.81	148.95± 11.73	52.90±1.25
ATTEB (150m/kg)	7.92±0 .37	6.78± 0.39	30.58± 1.43	18.81± 1.22	242.65± 22.49	127.87± 15.58	47.42±1.87
ATTEB (300m/kg)	9.91±0 .39	8.47± 0.41	32.02± 1.38	17.92± 0.83	317.81± 26.68	152.1±1 4.68	52.17±0.79

Carbon clearance Test (Phagocytic index):

The ATTEB high dose treated group showed considerable high phagocytic activity resultantly rapid carbon clearance from the blood. Improvement in phagocytic activity enhances body's immunity to kill and eliminate pathogens, thus, protecting against infections.

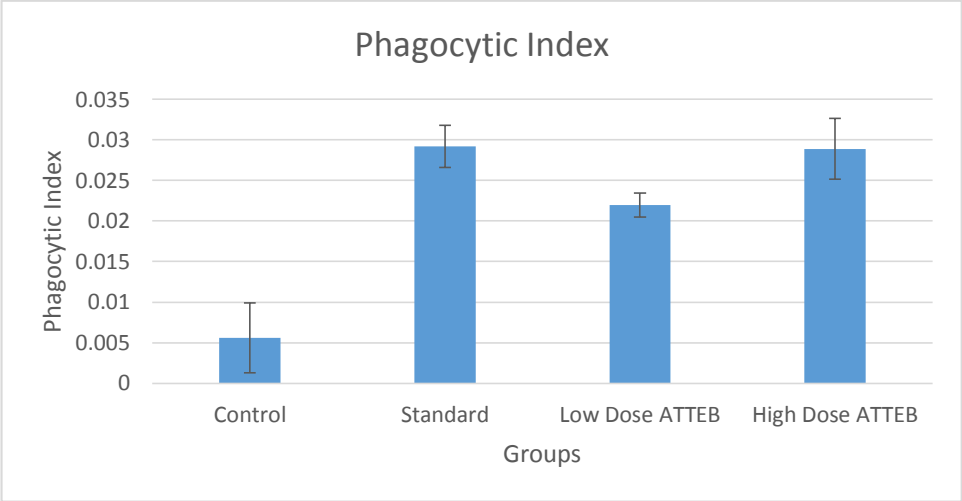


Figure 20. Phagocytic Index of control and treated groups. Value expressed as Mean ± STD. Data are considered statistically significant when $p < 0.05$.

Formulation Development:

Table 12. Effervescent tablet formulation table (To be dissolved in warm water before consumption):

Ingredients	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**	**B8**	**B9**	**B10**
Extract blend	250	250	250	250	250	250	250	250	250	250
Sodium bicarbonate	135	125	125	150	125	135	125	115	125	150
Tartaric acid	29	40	30	40	30	30	30	30	20	20
Lactose	40	39	49	14	49	49	49	59	59	34
Sucrose	30	30	30	30	30	30	30	30	30	30
Sodium benzoate	10	10	10	10	10	10	10	10	10	10
Polyvinylpyrrolidone	6	6	6	6	6	6	6	6	6	6
Total	500	500	500	500	500	510	500	500	500	500

Evaluation of formulations

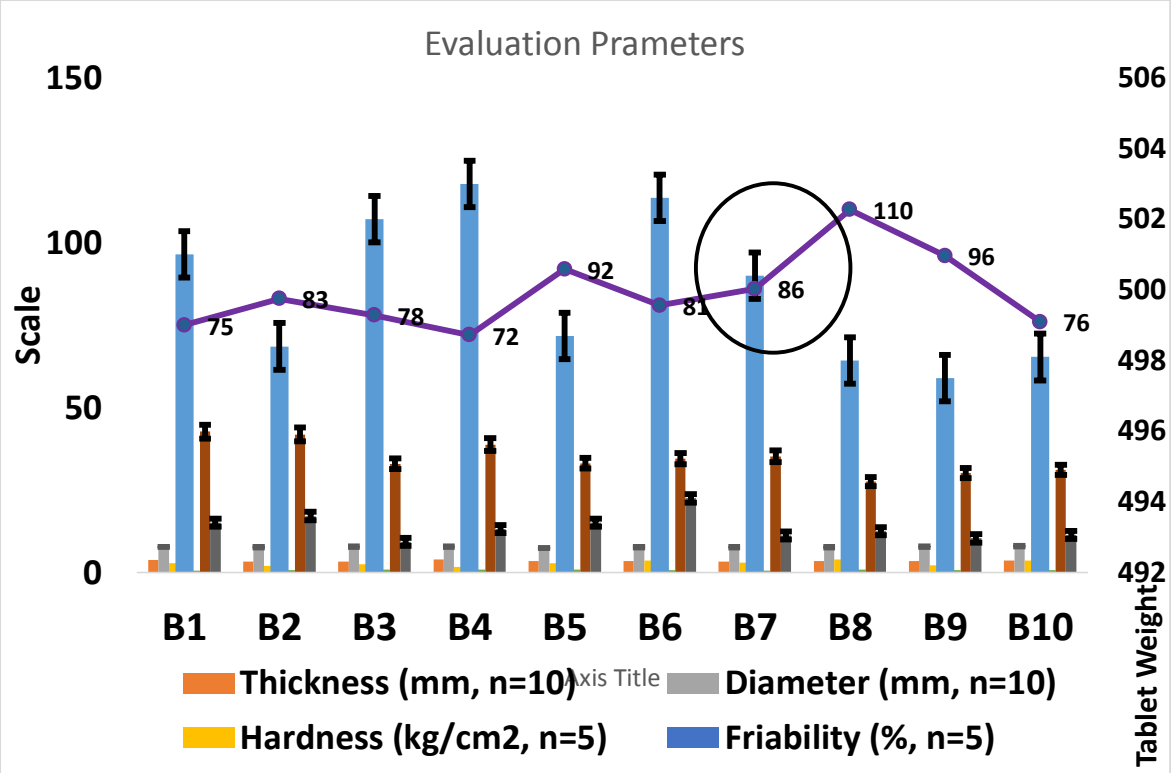


Figure 21. Evaluation of formulations. Value expressed as Mean ± STD. Data are considered statistically significant when $p < 0.05$.

Effervescent tablets were prepared as ready to use formulation.

Tablet evaluation parameters:

Following parameters were assessed: Weight variation, thickness, diameter, hardness, friability, disintegration time

The parameters complied as per the requirements of Indian Pharmacopoeia. Stability Studies of Effervescent Tablet

Figure 22.Effervescent tablets

Stability studies for formulation:

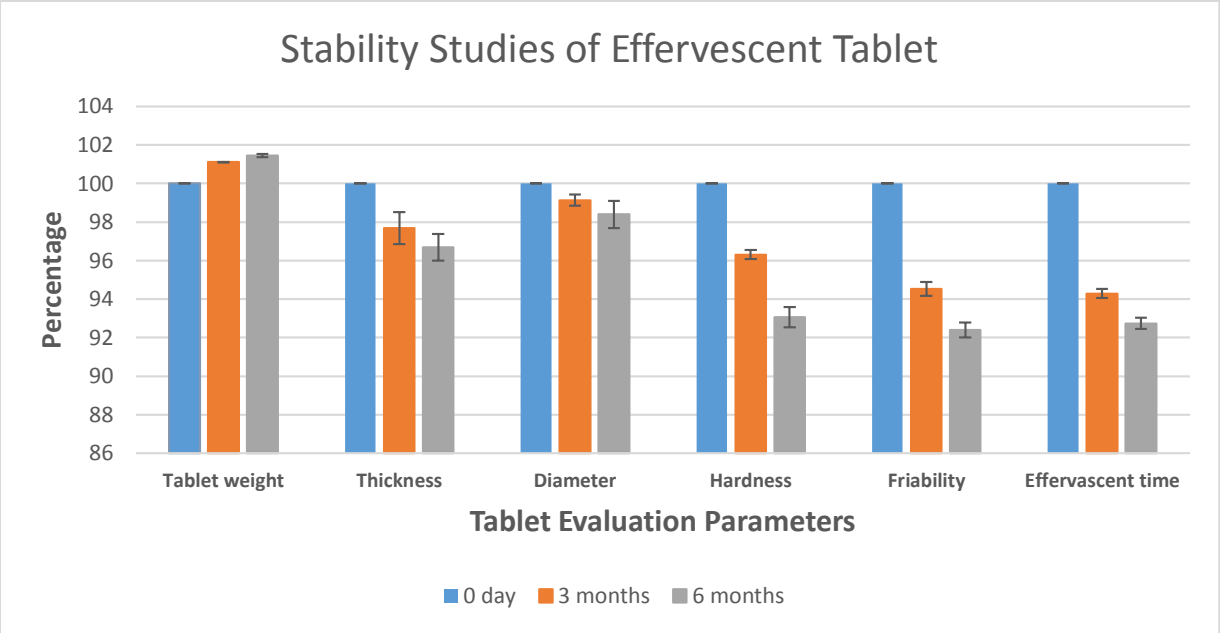


Figure 23. Stability Studies of Effervescent Tablet. Value expressed as Mean ± STD. Data are considered statistically significant when $p < 0.05$.

Formulation table for granule dosage forms:

Table 13. Formulation of granule dosage forms

S. No.	Ingredients (mg)	F1	F2	F3	F4	F5
1	Extract (Shatavari, Methi, Guduchi)	250	250	250	250	250
2	Citric acid	250	275	300	325	300
3	Tartaric acid	175	125	100	75	150
4	Sodium bicarbonate	375	400	400	400	350
5	Sucrose	28	28	28	28	28
6	HPMC in IPA 2%	22	22	22	22	22
	Total	1100	1100	1100	1100	1100

Evaluation of granule dosage forms:

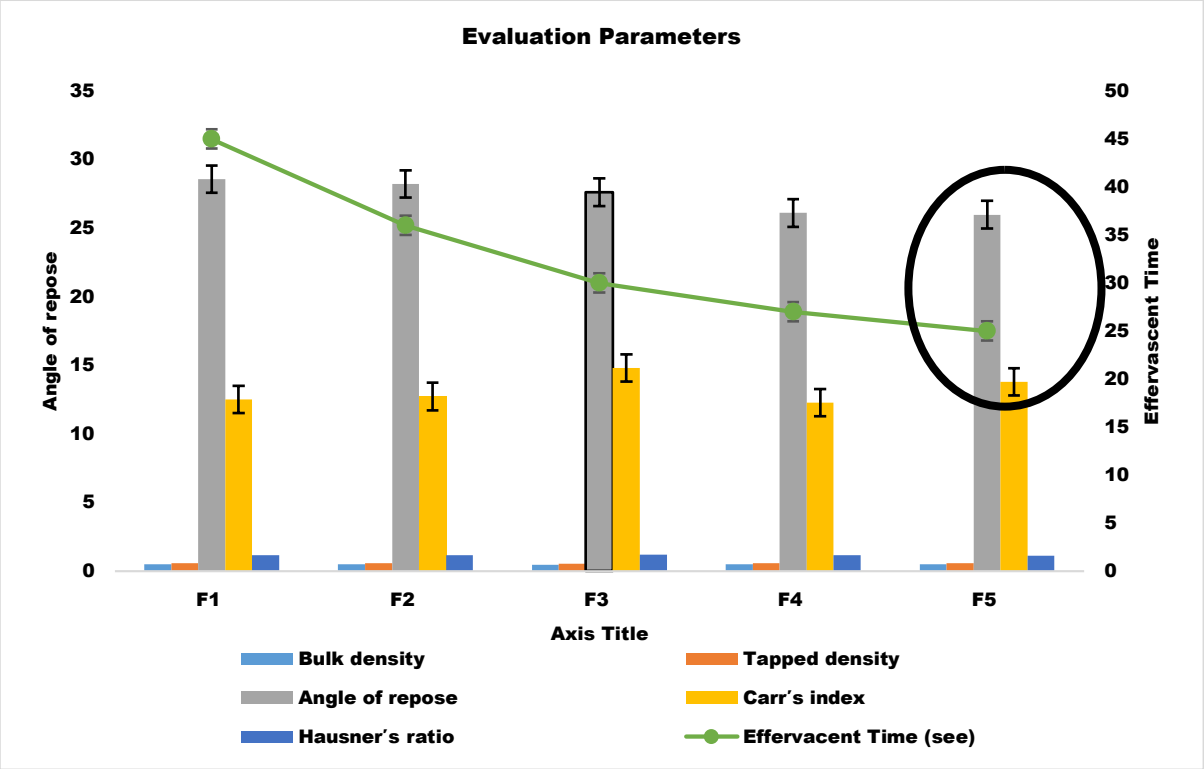


Figure 24. Evaluation of granule dosage forms. Value expressed as Mean ± STD. Data are considered statistically significant when p< 0.05.

Stability Studies of Granule Formulation

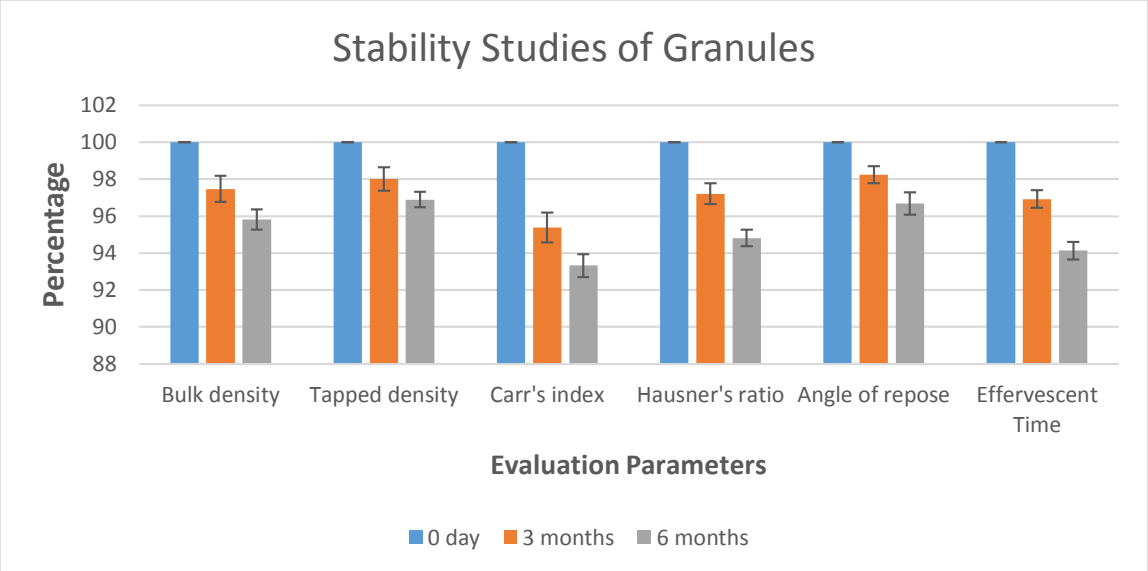


Figure 25. Stability Studies of Granule Formulation. Value expressed as Mean ± STD. Data are considered statistically significant when p< 0.05.

Discussion:

Hypogalactia is frequent in preterm and complicated delivery cases. Enhancement of milk in such cases is highly desirable to achieve the immediate and long-term well-being of the baby. Multiple practices and drugs in alternative therapies have been discussed in several research papers and traditional literature to accomplish this. Many of them are highly effective clinically and still prescribed frequently.

In the post COVID era, nutrition and immunity has gained global attention. It has been well recognized about infants that an initial immunocompetence and optimal nutrition lead to a higher sustainable and healthy life. Thus, finding novel ways of enhancing milk quality and quantity may be a more productive approach to afford better health and survival of infants. Various existing animal models to analyze lactogenic activity of chemical entities were reported employing Rat, Mouse, Goat, Sheep, Cow, Buffalo, Dog etc. [2122].

The current study evaluated possibility to employ rabbit for evaluation of Galactagogue activity of drugs. The study also evaluates herbal extracts of *A. racemosus, T. foenum graceum, T. cardifolia* for their galactagogue and immunomodulation activity with their acute and repeat dose toxicity. Effervescent tablet and effervescent granules formulation were developed with the same blend of the extracts.

To initiate the study, an analogy of basic molecular pathway for milk synthesis and secretion of rabbit with human was evaluated. For the preliminary evaluation, *in-silico* study was run and homology of gene nucleotide sequence, Prl receptor amino acid sequence and Prl hormone amino acid sequence of rabbit and human were aligned. For a comparison, similar studies were performed with rat Prl gene, Prl receptors and Prl hormone. The alignment revealed a remarkable similarity in Prl gene, Prl receptor and Prl hormone of rabbit with human. The similarity was higher as compared to rats. The outcome potentiated the hypothesis that molecular pathway of lactation of rabbit and human have similarity and a superior animal model over currently proven model animal.[23]

The lactation pattern of the rabbit, variation in milk nutritional component from 1st day to the 21st day postpartum, and effect of drugs on the same were analyzed. In the study, galactagogues, extensively reported in scientific literature, were used along with the control group to validate the effect on milk secretion and synthesis in rabbits. The galactagogue drugs used in allopathic medicine i.e., domperidone and metoclopramide with a well-established and scientifically reported ayurvedic galactagogue medicine widely employed in alternative and complementary medicinal system i.e., Shatavari, Methi, Giloe were used in the study.

Enhancement of milk production was found to be significantly high in all the treated groups compared to the control. The enhancement in milk production can be attributed to raised Prl level. The upsurge of Prl is in tune with the milk production in respective groups. The enhancement is found to be progressive with time. [24]

Furthermore, modulation in the synthesis of principal nutritional components of milk like protein, fats, solid non-fat, and minerals were analyzed. The study revealed that allopathic drugs, i.e., domperidone and metoclopramide enhance the overall volume of milk production along with minerals and lactose. Though, enhancement in other nutritional components of milk is insignificant in domperidone and metoclopramide treated groups. Herbal drugs enhanced the quantity of milk as well as the quality of milk i.e., protein, fats, mineral contents, solid non fats and milk immunoglobulins.

Currently, the preclinical studies for galactagogue studies are performed on rat and mouse models, which have never been reported to be validated and cross analyzed. These models have several limitations. Because of multiple unique features of rabbit, it serves to be more suitable animal model to study lactogenic effect of drugs. Rats and mice feed pups randomly throughout the day, thus, chances of error are more while recording the weight of pups and dams before and after feeding. To minimize these errors metabolic weight loss is required to be considered. [25] Uniquely rabbit does usually feed kits once or twice a day thus errors in recording actual weight gain can be minimized by fixing a particular time for feeding and weight recording. Due to diverse feeding habits, recording of weight gain due to milk consumption is difficult in guinea pig, ferrets, hamsters, and other laboratory animals of nearby phylogeny. [26]

To study the toxicity of the blend acute and repeat dose toxicity studies were performed as per OECD guidelines. The study revealed that the blend of the extracts does not have significant toxic effects. Thus, the blend of extract may be developed as a nontoxic galactagogue drug.

The quantity of milk samples collected from rats and mice is low to perform qualitative and quantitative analysis of milk nutrients. [27],[28] Thus, it facilitates the study of only modulation of milk production but not qualitative and quantitative analysis of milk nutrients. Contrary to this, collection of desired amounts of milk to perform qualitative and quantitative analysis of milk nutrients can be done with rabbit model.

To improve the ease of administration, improve shelf life, and improve efficacy etc., effervescent tablet and effervescent granules dosage form of the blended extracts were prepared. The formulation was found to be stable as per ICH guidelines. Thus, the drug was found to be a highly effective galactagogue and immunomodulator.

Summary and conclusion

- The extracts of Tinospora cordifolia, Trigonella foenum graceum, Asparagus racemosus were prepared and standardized. The extracts were found to comply with pharmacopoeial standards.
- The acute and repeat dose toxicity studies were performed as per OECD guidelines and the extracts were found to be non-toxic.
- The galactagogue activity was performed on Rabbit and extracts significantly enhanced milk secretion in rabbit.
- The extracts were studies for Immunomodulatory activity on mice and found to enhance phagocytic activity and stimulate neutrophil activation.
- Effervescent tablet and granule, as ready-easy to use product, were formulated and evaluated. The formulation qualified the standard evaluation parameters.
- Stability of the formulation was evaluated as per ICH guidelines and formulations were found be stable..

Awards:

1. **Best Paper Award** in symposium organized by RRCAT funded by Department of Atomic Energy (DAE, Government of India) & BRNS.
2. **Consolation Prize** for in Paper Presentation in AICTE Sponsored Online National Conference organized by SAIP, Indore.
3. Received '**SPARSH-2020' award** (Runner-up) by NITIE (Estb. by United Nations and Government of India) for extending Pharmacy Practice to remote rural areas.
4. Presented Paper at 8th Intl. Congress of the Society for Ethnopharmacology-India 2021.

Patent: Patent Granted. A POLY-HERBAL FORMULATION TO IMPROVE LACTATION". Australian application number- 2021107487

Publication: One Research and one Review publication under review process.

Start-up grant: Applied for a Start-up grant of Rs.15 lakh to IEDC, SVKM'S NMIMS (grant of MSME, Government of India) – Qualified in first round, under consideration for next level**.**

Industrial Collaboration: In discussion with Sambhav Pharma, Indore about Tech. Transfer.

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